MIGRATION STUDIES OF PLASTICIZERS FROM PVC FILM INTO FOOD

by

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ABSTRACT

Migration of plasticisers from thin flexible polymer films has been studied by a combination of laboratory migration experiments, analysis of retail food samples and mathematical modelling.

The main plasticiser studied was di(2-ethylhexyl)adipate (DEHA) but retail surveys also estimated consumer exposure to four phthalate ester plasticisers, acetyl tributyl citrate and dibutyl sebacate. Migration of these plasticisers was from polyvinyl chloride (PVC), polyvinylidene chloride (PVDC) and nitrocellulose-coated regenerated cellulose film (RCF) was determined by a stable isotope GC-MS technique.

The home use of DEHA plasticised PVC was simulated in laboratory experiments with real foods. In certain conditions the migration of DEHA was unacceptably high. The consequences of the introduction of low migration polyester plasticisers were determined by a comparison of their migration with DEHA. The method of analysis for trace levels of polyester plasticiser in food developed used a stable isotope dilution GC-MS technique and revealed migration levels of 3-20 times lower than DEHA.

The molecular size distribution of polyester plasticisers migrating in food was determined using size exclusion chromatography followed by GC analysis. The distribution of oligomers migrating into olive oil was determined and used in conjunction with DEHA migration data to successfully predict migration levels of polyester plasticiser into real food. The level of migration of individual oligomers was inversely proportional to their molecular weight.

A mathematical model of additive migration was evaluated for predicting DEHA migration from PVC into food. The diffusion coefficient of DEHA in cheese and the partition coefficient of DEHA between cheese and PVC cling film was determined. Using these values the model successfully predicted, to within a factor of 1·5, migration levels into fatty foods.

The determining factor in the level of DEHA migration predicted is the diffusion coefficient of DEHA in the food. The migration of DEHA from PVC into food was Class II in nature.

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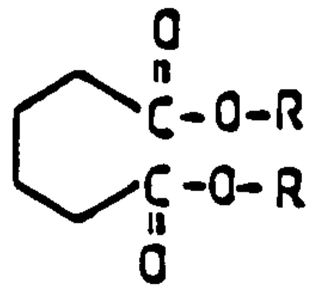
LIST OF ABBREVIATIONS

ACN	acetonitrile
ATBC	acetyl tributyl citrate
AAS	atomic absorption spectroscopy
BBP	butylbenzyl phthalate
BF_3	boron trifluoride
C	concentration of the diffusant
C_{Po}	original concentration of the additive in the polymer
D	diffusion coefficient
DBP	dibutyl phthalate
d_4-DBP	deuterated dibutyl phthalate
DBS	dibutyl sebacate
DCHP	dicyclohexyl phthalate
d_4-DCHP	deuterated dicyclohexyl phthalate
DCM/C_6H_{12}	dichloromethane/cyclohexane
DEHA	di(2-ethylhexyl) adipate
d_4-DEHA	deuterated di(2-ethylhexyl) adipate
DEHP	di(2-ethylhexyl) phthalate
DEP	diethyl phthalate
d_4-DEP	deuterated diethyl phthalate
DEHPim	di-(2-ethylhexyl) pimelate
D_f	diffusion coefficient of the additive in the food
DHP	diheptylphthalate
DMA	dimethyl adipate
DMPim	dimethyl pimelate
DOTEHT	di-n-octyltin-bis(2-ethylhexyl thioglycolate)
D_p	diffusion coefficient of the additive in the polymer
DPOP	diphenyl 2-ethylhexyl phosphate
ESBO	epoxidised soya bean oil
F	flux
GC	gas chromatography
GC-FID	gas chromatography with flame ionization detection
GC-MS	gas chromatography-mass spectrometry
HPSEC	high performance size exclusion chromatography
K	partition coefficient
$K_{DEHA\ cheese/film}$	partition coefficient of DEHA between cheddar cheese and PVC cling film

MAFF	Ministry of Agriculture, Fisheries and Food
MeOH	methanol
$\bar{M}_n$	number-average molecular weight
M_p	percentage oligomer migration
M_t	mass of additive migrated from the polymer in time t
$\bar{M}_w$	weight-average molecular weight
NTP	National Toxicology Programme
Na_2SO_4	Sodium sulphate
OCSEC	open column size exclusion chromatography
PVC	polyvinyl chloride
PVDC	polyvinylidene chloride
RCF	nitrocellulose-coated regenerated cellulose film
RH	relative humidity
R346	Reoplex 346 (Ciba Geigy)
RSD	relative standard deviation
SEC	size exclusion chromatography
t	time
US FDA	United States Food and Drug Agency
VCM	vinyl chloride monomer
V_i	inclusion volume
V_o	void or exclusion volume
x	distance

LIST OF MOLECULAR STRUCTURES OF THE COMPOUNDS OF INTEREST

Empirical Formula



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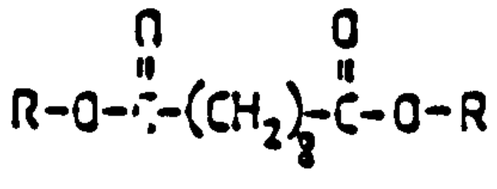

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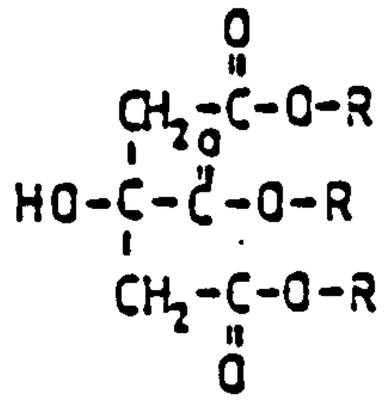
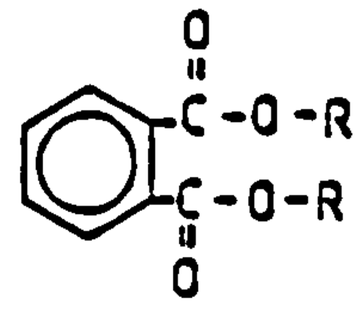


Citrate



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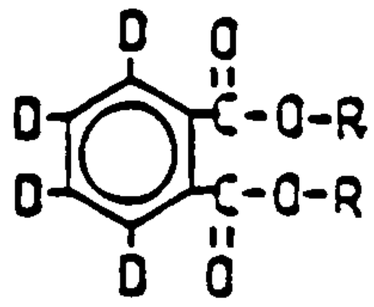

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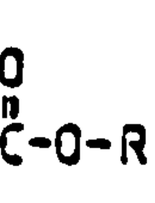
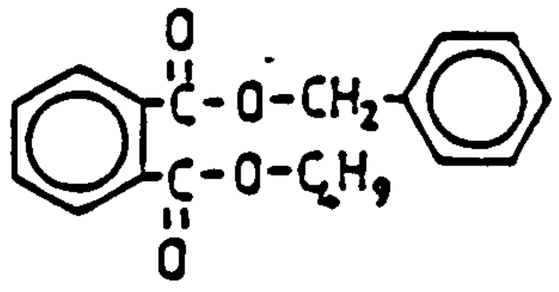


Sebacate

$CH_3OC(CH_2CO_2C_4H_9)_2CO_2C_4H_9$

Acetyl Tributyl Citrate



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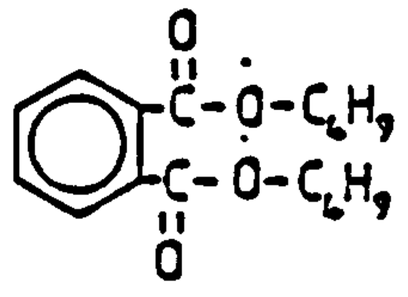

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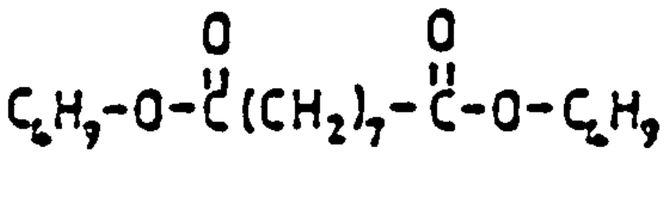

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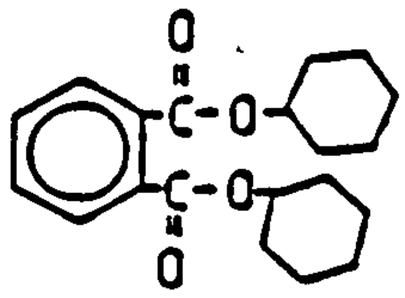

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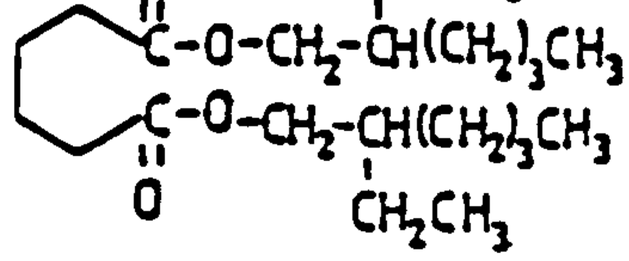

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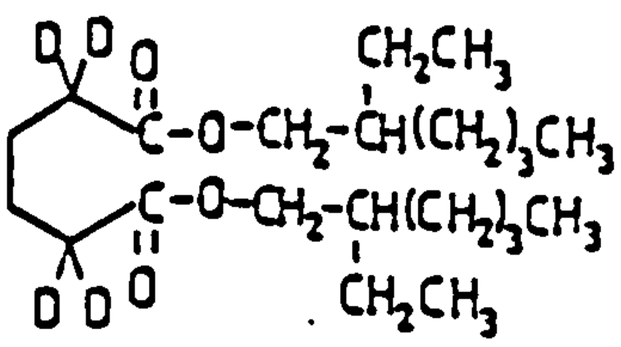

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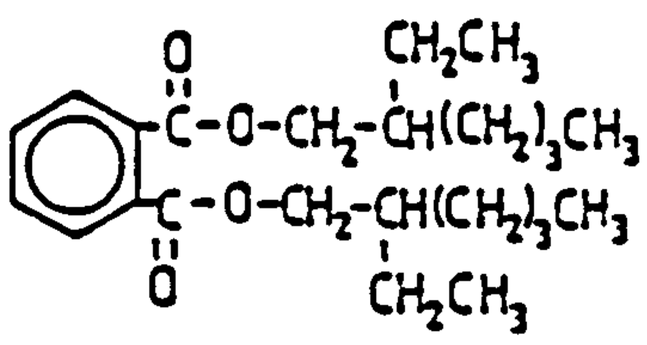

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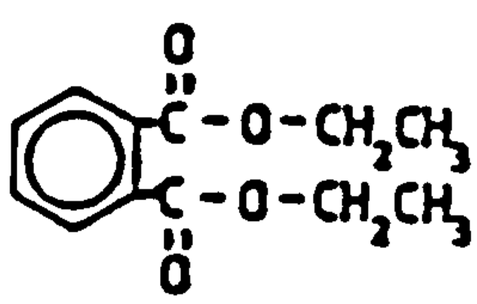
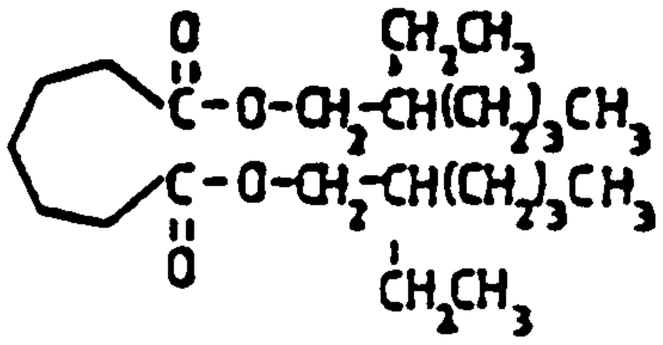


Diethyl Phthalate



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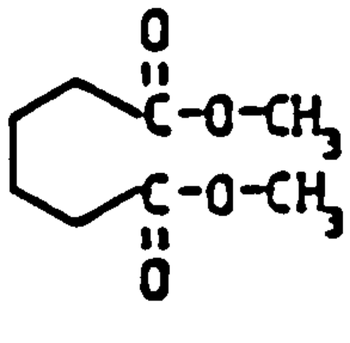

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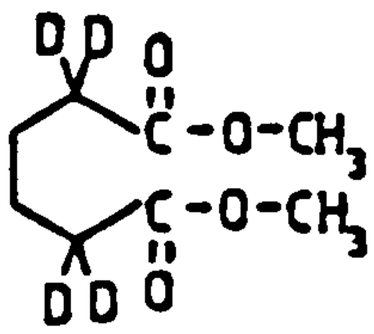

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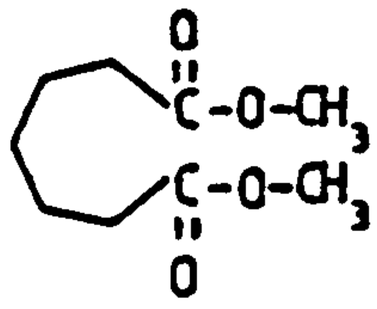


Dimethyl Pimelate

CHAPTER 1

LITERATURE REVIEW OF MIGRATION FROM PVC PACKAGING INTO FOOD

1.1 THE USE OF PLASTICS IN FOOD PACKAGING

The use of packaging to aid the preservation or transportation of food is by no means a modern phenomenon. Glass, earthenware, wood, cloth and leather were used by the earliest civilisations and canning, still an extensively used method of food preservation, was developed in about 1800.

In the last forty years, due to social and technological changes, the packaging of food prior to retail sale has become increasingly important. The UK packaging market grew by 300 % between 1971 and 1979 (1). The expansion of the packaging market can be clearly seen on the shelves of any modern supermarket by the variety of packaging materials used for the various products. These materials include paper and coated-paper products, cellulose products, cellophane, tinplate, aluminium, stainless steel, ceramics, glass, rubber, aluminium foil, plastics and laminates.

Since its introduction in about 1950 the use of plastic in the packaging industry has increased at a greater rate than other packaging material (2). Between 1971 and 1979 the UK market for plastic increased from £100 million to £746 million compared to £190 million to £650 million for tin plate, £94 million to £354 million for glass and £85 million to £226 million for paper in the same period (3).

A diverse range of polymers are used in packaging reflecting the many different requirements of modern food packaging. Those most commonly used include poly(ethylene), poly(propylene), poly(vinyl chloride) (PVC), vinylidene chloride/vinyl chloride copolymer, poly(ethylene terephthalate), poly(styrene), and styrene/acrylonitrile/butadiene copolymers.

When selecting a polymer to use in food packaging a number of factors have to be considered including the possibility of migration from the polymer into the food. Although the base polymers are generally considered inert, all plastics packaging contain a variety of low molecular weight components that could potentially migrate into the food. These low molecular weight species may include residual monomers, oligomeric material, solvents, catalyst residues, additives such as: heat and light stabilizers, antioxidants, plasticisers, colourants, U.V. absorbers; processing aids such as: lubricants, slip agents, blowing agents, emulsifiers, defoamers, antistatic-, antislip- and antifog agents, and inorganic fillers and reaction and decomposition products. Trace impurities in the polymer starting materials and additives, along with breakdown products from the additives, add to the list of potential migrants.

Migration between food and packaging materials can have a number of important consequences. The nature, quality or substance of the food may be altered which can give rise to problems of food safety or quality. The loss of food constituents such as aroma and flavour compounds into the packaging material can produce organoleptic imbalance in the packaged food. And the migration of food constituents

into the packaging material can, for example adversely effect the physical or chemical properties of the packaging impairing its barrier or sealing properties.

This review will consider the implications of migration from plastic packaging into food from a food quality stand point. It will concentrate on food safety as the tainting of food by plastic packaging may be regarded as self regulating. If, as the result of a product being packed in plastic, off-flavours or aroma imbalance develops, the consumer will not purchase the product. The manufacturers will then take remedial action thereby resolving the problem.

Migration from plastic packaging into food has been a very active research field since the late 1960's. A recent review of the determination of plasticisers in food, a single class of migrant, cited 154 references between 1967 and 1986 (4). It is clearly beyond the scope of this review to exhaustively consider such a large body of work.

This review is restricted to migration from PVC, which is the polymer considered in this study and is the most widely used, after poly(ethylene), polymer in food packaging (5). It is further restricted to the migration of monomers, organotin stabilisers and plasticisers. The use of food simulating solvents in the determination of migration from plastic packaging into food is also briefly considered, as food simulating solvents have played a major role in the determination of migration from plastic packaging into food.

The studies highlighted have been selected to illustrate the range of analytical techniques employed for determining migration from plastic packaging at trace (mg kg^{-1}) and ultra-trace (µg kg^{-1}) levels in complex food matrixes. Selection has favoured those methods employing modern instrumental techniques of chemical analysis and also reflect, to some extent, the author's particular interests.

1.2 USE OF FOOD SIMULATING SOLVENTS IN THE DETERMINATION OF MIGRATION FROM PLASTIC PACKAGING INTO FOOD

Most foodstuffs are complex matrixes consisting of a mixture of water, fat, proteins, carbohydrates, vitamins and minerals. The potato, which is 76% water, contains at least 150 chemical entities (6), 98 volatile components have been identified in blackcurrants (7) and orange oil is known to contain at least 42 different chemical species (8). The chemical composition of a food may also differ according to the variety of species, soil conditions, and climate. In addition to the chemicals that naturally occur in foodstuffs, preservatives, antioxidants, stabilisers, flavours, sweeteners or natural colours may be added when the food is processed. The Handbook of Food Additives lists 1,697 food additives (9).

Food simulating solvents, often referred to as food simulants, have been developed due to the inherent difficulties, as illustrated above, of analysing for a migrating species in food at the trace or ultra trace level. The migration of plastic components into actual foods other than vegetable oils has been measured infrequently. Occasionally

the use of a food simulant is the only practical approach to assessing the migration of an additive from the packaging into the food.

Ideally a food simulating material should adequately reflect the food's chemical and physical properties and fulfil the following criteria. The level of migration from the plastic into the simulant should be similar to that into the food it is simulating and the food simulant should permit a relatively simple method of analysis for the migrant of interest (10). All of the simulants proposed to date are to some extent compromises between these two conflicting requirements.

In order to simplify the selection of suitable food simulating materials foodstuffs have been divided into the following categories, dry solids, neutral liquids, acidic, ethanolic or fatty substances.

The majority of work on the development of food simulants has been devoted to simulants for neutral liquids, acidic, ethanolic and fatty foods. This may be due to an assumption that migration into dry foods such as, milk powder, dehydrated soup, flour and cereals, is minimal and therefore of little concern. Nevertheless, a number of simulants for dry foods have been proposed, including activated carbon (11), Kieselguhr impregnated with a known mass of the test fat HB307 (12) and celite impregnated with a known mass of olive oil (13). Schwope and Reid reported that migration plastic packaging into dry foods does occur. The rate of migration being determined by the intrinsic diffusion coefficient of the additive in the polymer (11).

There appears to be little debate as to suitable simulants for neutral liquids, acidic and ethanolic foods. Migration into foods of pH 5 and above may be simulated with distilled water. In the case of fish it may be appropriate to add sodium bicarbonate to the distilled water as packed fish can contain high concentrations of ammonia and substituted amines. Acidic foods (pH of less than 5) such as vinegar, pickles or fruit juices may be simulated by aqueous solutions of between 2% and 5% acetic acid. Migration into alcoholic foods may be simulated by solutions of between 5% and 50% ethanol in distilled water, 5% for beers and ciders, 15% for table wine and up to 50% for spirits. Selection of the appropriate food simulant must be considered with some care. Distilled water, for example, is an inadequate simulant for milk (14).

A completely satisfactory simulant is not available for fatty foods. Two categories of simulants have been proposed. Organic solvents such as: ethanol, liquid paraffin, n-heptane (15), diethyl ether (16) and isooctane and edible fats such as: coconut oil, olive oil, sunflower oil, lard and a mixture of synthetic triglycerides (17).

Migration into the simple organic solvents tends to be markedly different to that into food. This is due to a tendency of the organic solvents to cause excessive swelling of the polymer which accelerates the migration of the additive. Migration into n-heptane was found to be more rapid and extensive than occurs into fatty foods and making n-heptane an unsuitable simulant for fatty food (18). De Kruijf has extensively studied the use of isooctane as a fatty food simulant in the determination of the total migration of components from plastics

into food. De Kruijf reports that the total migration from plastic packaging exposed to isooctane for two hours at 40°C or two days at 20°C is equivalent to that observed for plastic exposed to the commonly used fatty food simulants for ten days at 40°C (19). Therefore isooctane may be used in a simple and rapid test to assess the total migration from plastic packaging into fatty foods.

The use of edible fats as fatty food simulants has been more successful than organic solvents but is not entirely satisfactory. The levels of migration into edible fats is not always representative of the migration into fatty foods and the use of edible fats can present nearly as many analytical difficulties as the fatty foods.

The Commission of the European Community has specified the following simulants for use in tests to ensure that food contact plastic fulfils certain directives regulating permissible migration from the packaging into food (20,21,22). Distilled water or equivalent, 3% (w/v) acetic acid in aqueous solution, 15% (v/v) ethanol in aqueous solution and rectified olive oil (23). However, due to the difficulties associated with the use of olive oil a standard mixture of synthetic triglycerides or sunflower oil may also be use as a fatty food simulant.

In an attempt to overcome some of the difficulties associated with the use of olive oil, which is a natural product with all its inherent complexity and variation, Figge developed a mixture of synthetic triglycerides referred to as HB307 (24).

The fatty acid composition of HB307 is similar to coconut oil and therefore simulates the maximum migration that would occur into a fatty food. Figge *et al.* have used HB307 and radiolabelled HB307 extensively to study the total migration of components from plastic packaging into food and the migration of fat into plastics (25,26). However, HB307 is not widely used as fatty food simulant due to its cost and the regulatory requirement of the European Community to use olive oil (23).

1.3 VINYL CHLORIDE MONOMER

In 1973 Schenley Distillers reported finding vinyl chloride monomer (VCM) in alcoholic beverages, such as vodka and gin, which had been packaged in PVC bottles (27). As VCM is a human liver carcinogen (28) these findings had important implications for food safety as PVC was extensively used for food packaging.

In 1979 the European Community introduced restrictions on the amount of permitted VCM in food contact plastics and food. Not more than 1 mg/kg of free VCM should be present in a finished article and the concentration of VCM in food in contact with the article must not exceed 0·01 mg/kg VCM in food (29).

The introduction of this regulation provided a clear requirement for methods of analysis for VCM in packaging and foodstuffs. A wide range of colorimetric, electrometric and spectrophotometric techniques have been proposed for the detection of vinyl chloride monomer (30,31)

however gas chromatography (GC) has been the most widely used technique.

Two approaches have been adopted to the GC analysis of VCM in food, direct injection of a sample solution onto the GC and headspace analysis of the sample. In general headspace GC techniques gave lower detection limits than direct injection (32). The low boiling point of vinyl chloride, -14°C, makes it readily amenable to headspace GC analysis however, this coupled with VCM's insolubility in water, causes difficulties in the preparation of standards. Crosby (33) and Lande (34) have both extensively reviewed the difficulties associated with the preparation of VCM standards. Commercially prepared standards are available.

Williams and Miles used a direct injection method for aqueous samples and a headspace method for samples of peanut oil. A detection limit of 10-15 ppb was attained by direct injection and 5-10 ppb by headspace sampling (35). Williams also measured VCM in alcoholic beverages and vinegar with mass spectral confirmation, monitoring at *m/e* 62. The mass spectrometry detection limit was 10 ppb. Williams found that the recovery of the method was dependant on the food matrix. For example 65% of added VCM was found in vinegar, 60% in sherry and 50% in gin and Martini (36).

Diachenko *et al.* measured the migration of VCM into food simulating solvents and corn oil (37). A headspace GC procedure was used. A sample of PVC sheet was placed in a sealed vial containing the food simulant. Standard solutions of 1 ppb (wt/wt) VCM in each food

simulant were prepared and treated identically to the samples. The samples were stored at 49°C for either 19 or 54 days after which time aliquots of the samples were transferred to nitrogen purged vials. The vials were brought to 90°C and held at that temperature for 30 minutes before a 3 or 4 mL sample of the headspace was injected directly on to the GC. The detection levels ranged from 0·1 ppb (wt/wt) in 50% ethanol to 0·5 ppb (wt/wt) in corn oil.

The level of VCM migration into the food simulants was found to be dependant on the initial concentration of VCM in the polymer. VCM migration from the PVC ranged from two-thirds to near complete loss depending on the food simulant and length of exposure. The maximum loss was observed into corn oil that had been exposed for 54 days at 49°C.

A number of other studies on the migration of VCM from plastics into food and food simulating solvents have been reported (38,39,40). Generally most methods based on headspace GC analysis can detect levels of VCM down to 100 ppb in polymers and 1-5 ppb in foodstuffs.

Dennison *et al*. developed a technique to determine VCM at the 1 ppb (wt/wt) level in PVC articles utilizing a combination of headspace sampling and sparging (41). The sample is dissolved in dimethylacetamide and the VCM purged from the solution with helium for approximately two hours. The VCM is trapped in ethanol cooled by a dry ice/solvent mixture. The ethanol solution is then analysed by headspace GC with flame ionization detection and confirmation by GC

mass spectroscopy. This pre-concentration step enables low levels of VCM to be detected and some interferences to be removed.

The Ministry of Agriculture, Fisheries and Food established a Working Party on Vinyl Chloride which measured the level of VCM in PVC packaging and food packaged in PVC between 1974 and 1977. They found that in order to obtain reproducible results at low VCM concentrations, the 10 ppb level, automatic headspace sampling was desirable. Using such a technique they reported the following detection limits, aqueous medium 2 ppb, ethanolic medium 5 ppb, oleaginous medium 5 ppb and polymer (dissolved in a suitable solvent) 100 ppb (42).

The Working Party wanted to determine the factors affecting the level of free VCM in plastic packaging and assess the level of VCM in food thereby assessing the health risk VCM posed in food. To this end the level of VCM in 850 items was measured during the period 1974 to 1977. The items examined were representative of typical food use of PVC, that is bottles, rigid film, flexible film, bottle closure liners and heat seal aluminium foil.

The level of VCM in flexible films was always less than 1 ppm. The level of VCM found in bottles, heat seal aluminium foil, raw polymer, powder blend and virgin package all decreased throughout the period of the study. The level in a bottle wall decreased from 150 ppm to 5 ppm. The decrease of residual VCM in the packaging produced a corresponding decrease in the level of VCM found in the foods, such as fruit drinks, cooking oil, butter and soft margarine. In the early stages of the

study levels, of 0·1 to 1·0 ppm in food were found. At the end of the study all food samples contained less than 0·001 ppm and the majority contained less than 0·5 ppb. The corresponding calculated maximum likely irtake per person was no more than 1·3 µg/day in early 1974 and by may 1976 this had fallen to a maximum likely intake of 0·1µg/day.

The level of residual VCM in the fabricated article was influenced by the manufacturing process. Thus a reduction in the residual concentration of VCM in the packaging could be, and was, achieved by changes in the manufacturing processes. For example the hot blending manufacturing process used in the UK, significantly reduced the level of VCM in the manufactured article compared to the polymer just before blending.

1.4 ORGANOTIN STABILIZERS

Organotin stabilizers are added to PVC to limit thermal degradation and prevent discoloration at the high temperatures that PVC melt is normally processed. The stabilizers are believed to react with any hydrogen chloride present in the polymer and thereby prevent further degradation. The chemical reaction mechanism of organotin stabilisation of PVC and the performance of the various organotin stabilizers available have been reviewed elsewhere (43,44,45).

A variety of techniques have been used for the analysis of organotins in foodstuffs. These include colourimetry, atomic absorption spectrophotometry, anodic stripping voltammetry, and radiochemistry.

The most commonly used technique is colourimetry. The method typically involves an oxidative destruction of the food with a mixture of nitric and sulphuric acid followed by the addition of hydrogen bromide and hydrogen chloride to form a tin halide. This is then distilled and the concentration of tin determined colourimetrically (46). If food simulants are used in the migration experiments the distillation step can sometimes be omitted. The method is sensitive to 1-5 µg of tin depending on whether pyrocatecholsulphone phthalein (47) or 3,4-dimercaptotolunen (48) is used in the colourimetric determination.

Adcock and Hope (49) were able to achieve a detection limit of 0.2µg of tin by separating the tin complex from excess reagent using paper chromatography on asbestos or cellulose. The reagent used in the colourimetric determination was pyrocatecholsulphone phthalein.

The migration of a range of organotin stabilisers from PVC has been studied using anodic stripping voltammetry. The detection limit of the method developed was approximately 3·4 x 10^{-7}M (50).

Figge *et al.* carried out an extensive series of experiments investigating the migration of organotin stabilisers from PVC into foods and food simulants using radiolabelled organotin stabilizers (51-56). The results of these studies are discussed later in the review. Arthur D. Little, Inc. also successfully used a radiolabelled organotin stabiliser, isoctylmercapto-acetate, to study the migration of organotin stabilisers from PVC into food simulating liquids and food products (57). An advantage of the use of radiolabelled organotin stabilisers is that migration into complex matrixes, such as edible

oils, can be determined at low levels. A disadvantage is that the PVC packaging has to be specially prepared and therefore may not be truly representative of the packaging that comes in contact with the food.

Flame atomic absorption spectroscopy (AAS) has also been used to study the migration of organotins from PVC into water and edible oils (58). The water extracts were injected directly and oils samples diluted with cyclohexane. The limit of detection reported was 1·8 µg tin/g in PVC and approximately 10 ng tin/g in food simulants.

Dietz *et al.* also used AAS to study the migration of organotins from PVC pipes into water (59). Samples of test pipes were placed into glass jars filled with deionised water. The samples were stored for 8 weeks at 25°C and 50°C with sub-samples being taken each two weeks

The level of tin extracted from the pipes was low, the highest levels occurring within the initial two weeks. There was little different in the level of migration at 25°C of di methyl- and butyl- tin stabiliser however, octyltin migration was much lower. The data at 50°C showed the same trend, that is high initial migration levels followed by a decrease in the levels of tin detected with time. This phenomenon was attributed to the conversion of extracted tin components to an insoluble form and subsequent precipitation or absorption of the insoluble derivative on the walls of the jar or pipe. This hypothesis was confirmed by acidifying the extraction solution with concentrated hydrochloric acid before AAS analysis. This resulted in an eightfold increase in the levels of tin detected.

The US FDA has approved the use of di-n-octyltin-bis(2-ethylhexyl thioglycolate) (DOTEHT) and di-n-octyltin maleate polymer in food contact PVC and vinyl chloride copolymer (60) and set a maximum permissible level of octyltin migration into food at 1 ppm (61). A number of studies of the migration of these stabilisers have been reported, the majority of which have concentrated on the migration of DOTEHT.

Carr measured the level of organotin into a variety of liquid foods stored for two months at 30°C in PVC bottles containing DOTEHT and glass bottles (62). The level of tin in the samples stored in PVC was comparable to that found in the sample stored in glass. Indicating that there was no appreciable migration from the PVC and that correction for background level of tin is essential, if the true level of migration is to be determined.

Measurable levels of organotin migration have been reported for samples stored for longer than two months. Sunflower oil stored for six months in PVC bottles contained 2 ppm DOTEHT (63) and beer stored for eight months at 20°C contained 0·01 mg/L of DOTEHT (64). The migration of both DOTEHT and di-n-octyltin maleate polymer into a variety of foods stored for eight weeks at 57°C has been reported. The levels found ranged from 0·01 to 0·24 mg organotin/kg of food (65). These levels were not exceeded when the food was stored for one year at room temperature. Brighton reported that organotin stabilisers migrated readily from PVC food containers into olive oil, orange or lemon soft drinks and 50% aqueous ethanol (66).

Serval studies have used food simulating solvents to investigate the migration of dioctyltin stabilisers from PVC. The migration of DOTEHT from impact resistant PVC into seven simulants ranged from 0·004-0·045 mg octyltin/dm^2 polymer. The highest level of migration being into heptane (67). Keller also found the highest level of migration was into heptane when he compared the level of migration into foodstuffs and food simulants (68). The level of DOTEHT ranged from 0·06 mg/kg for 3% acetic acid to 0·44 mg/kg for heptane. There is evidence to suggest that heptane is not a very good simulant for fatty foods as workers have reported being unable to detect DOTEHT migration into coconut oil, triacetin or triglyceride mixtures (69).

Figge studied the migration of the organotin stabiliser ADvastab TM 181 FS, incorporated into rigid PVC film at the 0·5, 1·0 and 1·5% by weight level, into the fat simulant HB307. The stabiliser contained either methyltin-tri, dimethyltin-di or trimethyltin-thioglyolic acid-2-ethylhexyl ester which was labelled with ^{14}C in either the methyl or thioglycolte residues. Film samples (10 dm^2) were immersed in the fat simulant for 30 minutes at 70°C or put in one sided contact with 1 kg of fat simulant for 30 days at 20°C and 65% RH or 10 days at 40°C and 56% RH. The residual level of stabiliser in the contact film after exposure was independent of the initial concentration for samples exposed to identical storage conditions. Extending the length of exposure did not noticeably reduce the level of residual stabilisers in the PVC.

It has been suggested that organotins migrate into hexane and fatty foods while degradation products of organotins tend to migrate into

aqueous foods and food simulants. However the picture is far from clear. Woggon reported that di-n-octyltin esters do not decompose (70). Yen (71) found that the un-degraded organotin migrated into hexane and lipid-based simulants while a cyclic trimer organotin oxide $(R_2SnO)_3$ migrated into aqueous simulants. Small amounts of the un-degraded organotin and an alcohol form, $R_2Sn(OH)_2$, were also present in the aqueous extracts. However experiments with radiolabelled DOTEHT indicated that DOTEHT and its hydrolysed form migrate at the same rate into edible oils (72)

These findings were in agreement with the work of Figge *et al*. They studied the migration of radiolabelled DOTEHT and its three decomposition products 2-ethylhexyl thioglycolate, di-n-octyltin dichloride and di-n-octyl tin oxide into edible oil and HB307. They found that both the organotin and decomposition products migrated into the oils and HB 307.

Sheftel reported that the chemical nature of organotin stabilisers had little effect on their migration from compounded PVC and water (73).

The mechanism of organotin migration appears to have been established. Several studies present evidence that organotin stabilisers are extracted primarily from the surface of rigid PVC and not from the bulk polymer. Downes found rapid initial migration into heptane and 95% ethanol followed by a period of further migration at a decreased rate (74,75). The initial amount of migration was greatly retarded when the PVC surface was cleaned with heptane prior to extraction. If

the polymer was aged for two weeks following the surface cleaning the retardation was no longer observed upon extraction.

As indicated earlier, food grade PVC packaging contains a large number of different additives. Some workers have looked at the effect of other additives present on the migration of organotins. The migration of dioctyltin and dibutyltin compounds from PVC into 50% ethanol, 5% acetic acid and sunflower oil stored for ten days at 45°C appeared to be unaffected by the presence of up to 3% of the secondary plasticiser epoxidised soybean oil (76). Concentrations of up to 11% of epoxidised soybean oil had no effect on the global migration from rigid PVC into 10% aqueous ethanol or 3% acetic acid (77).

1.5 PLASTICISERS

Plasticisers are added to polymers to reduce their brittleness and increase their flexibility or workability. Of the large number of plasticisers available (480 in 1981, (78)) approximately 100 are of commercial importance (79). These are typically high molecular weight esters such as phthalic acid esters, often referred to as phthalates. Phthalates are the most extensively used plasticisers, in 1979 they accounted for over 60% of all plasticisers produced in the USA (80), and have become ubiquitous in the environment.

The majority of studies of plasticiser migration have concentrated on the migration of phthalates into food simulants. The complex nature of

food and the extensive contamination of the environment by phthalates has made the analysis for phthalates in foodstuffs very demanding.

Some of the earliest studies focused on the migration of di(2-ethylhexyl) phthalate (DEHP) from PVC milk tubing into milk. DEHP is particularly insoluble in water but soluble in fat (81) and therefore it is found in milk (82).

A number of techniques have been used for the analyse of DEHP in milk. The methods generally involve an organic solvent extraction, a clean-up step to remove the co-extracted lipids, separation of the plasticisers and then quantification by thin layer chromatography (83), spectrophotometry (84) or GC (85). Some methods include a saponification step to convert the phthalates present to either phthalic acid (84), thereby determining total phthalate content, or 2-ethylhexanol (85). A variety of solvents have been used in the extraction stage including petroleum ether (86), diethyl ether-petroleum ether (87) and methanol-diethyl ether (85).

Reported levels of DEHP in milk varied greatly. Leoni *et al.* found levels of between 2 and 600 ppm DEHP (86) and Cerbulis *et al* found 80 ppm DEHP in milk (83). The Federal Republic of Germany has restricted the DEHP content of PVC milk tubing to 10% (88).

A small number of studies have determined the migration of phthalates into foodstuffs. Methods of analysis used can be summarised in the following steps. Maceration of the sample, extraction into a polar solvent and then partition the plasticiser into a hydrocarbon solvent

with water. Quantification is then by GC with flame ionization detection or electron-capture detection. Some of the later methods use gel permeation chromatography as a clean-up step to separate the co-extracted food components from the phthalates (89,90,91). A range of extracting solvents have been used depending on the foodstuff of interest and include methylene chloride (92), acetonitrile (93, 94), diethyl ether (95), acetone-diethyl ether (96), hexane (97,98), and acetone-hexane (89).

The limit of detection of the various methods used was dependant on the food matrix. For non-fatty foods such as fish, shrimps, crab, soya sauce and jam a detection limit of 1 ppb was typically obtained. For fatty foods such as duck, edible oils, meat pies and confectionery detection limits of 0·1-0·5 ppm were reported.

A number of national surveys of phthalate contamination of food have been reported. Tomita *et al*. examined 55 samples of Japanese food and found levels of dibutyl phthalate (DBP) and DEHP of between 0·01 ppm and 26 ppm (99). Powdered and granular foods showed the highest level of migration with a sample of frying powder (tempura) stored for 8 months containing 430 ppm. Cocchieri surveyed 200 Italian foods for DEHP and DBP (100). In general the levels of plasticisers in the foodstuffs were low with three exceptions. Salted meat contained 6·7 ppm DEHP and cheese and potato chips contained 17·5 ppm and 12·0 ppm of DBP respectively.

Castle *et al* surveyed the migration of phthalates into foodstuffs from printing inks and films used for retail food packaging in the UK

(91,101). Levels of DBP, DEHP and dicyclohexyl phthalate (DCHP) due to printing inks varied with food type. Chocolate and chocolate sweets contained 0·3-9·2 ppm DBP and 0·06-2·4 ppm DEHP, potato crisps and snack products contained 0·1-14·1 ppm DBP, <0·01-18·6 ppm DCHP and <0·01-1.8 ppm DEHP. The levels of plasticisers found in retail foods wrapped in phthalate plasticised films were similar to the above results. The foods were all wrapped in nitrocellulose coated regenerated cellulose film. Confectionery contained 0·5-30·8 ppm DBP the highest level found being in chocolate, meat pies and pasties 1·2-15·6 ppm DBP, 0·2-16.9 ppm DCHP, and 1·2-12·0 ppm butyl benzyl phthalate (BBP), sandwiches 12 ppm DBP, 15 ppm DCHP and 14 ppm BBP and baked products contained approximately 0·5 ppm of diethyl phthalate.

PVC plasticised with di-(2-ethylhexyl) adipate (DEHA), commonly known as cling film, is widely used as a food wrap as it is flexible, transparent and has a high permeability to oxygen. Analysis typically involves an organic solvent extraction and quantification by GC or GC-mass spectrometry (102, 103). One of the reported methods includes a clean-up step using gel permeation chromatography to separate the co-extracted lipids from the DEHA (104).

The level of DEHA found in the cling film ranged from 13% to 26% by mass. Migration was most extensive into high fat foods such as cheese, 1,300 ppm (103) and high fat content meats (105), 23·5 $mgkg^{-1}$. Tatsuno *et al*. reported that DEHA did not migrate into the food simulants 4% aqueous acetic acid or distilled water. This finding was consistent with the reported levels of DEHA in high water, low fat content foods such as fruit, vegetables and certain cakes (106,107).

Vinylidene chloride (PVDC) copolymerised with PVC is plasticised with acetyltributyl citrate (ATBC) producing a film which is widely used for the retail packaging of cheese and is recommended for the covering of food during domestic microwave cooking.

Two methods are available in the literature for the analysis of ATBC. One involves a diethyl ether extraction followed by saponification, distillation, a second diethyl ether extraction and GC analysis (95). The second is a stable isotope dilution technique. A deuterated internal calibrant is added to the sample which is homogenised, extracted with acetone/hexane, the co-extracted lipids separated from the ATBC by gel permeation chromatography and the concentration of ATBC present determined by GC-mass spectrometry (108).

The data available on the migration of ATBC into food is very limited. Motegi *et al.* reported levels of 30-160 $mgkg^{-1}$ in edible oils and fatty food simulants exposed to film for 90 minutes at 90°C (109). Reported levels of migration into foods are lower. Cheese exposed to PVDC/PVC film for several days contained 1·3-7·7 $mgkg^{-1}$ (101). Chicken breasts and a variety of other foods cooked in a microwave oven in contact with film contained between 0·4-35·0 $mgkg^{-1}$ (110).

There is a certain amount of uncertainty as to the toxicity of plasticisers, and thus the health risk of plasticisers migrating into food is also unknown. Concern was initially expressed at the level of DEHP and DEHA migration into blood. Studies showed up to 50-100 mgL^{-1} of DEHA and DEHP migrated from PVC biomedical products into blood (111). During a six hour period approximately 60 mg of DEHP was

extracted from a complete haemodialysis tubing set (112). Although no toxic effects were observed, it was recommended that alternative tubing with no extractables should be developed and used (113).

This recommendation had important implications for the use of DEHP and DEHA as plasticisers in food packaging. In 1981 the National Toxicology Programme (NTP) in the USA published results indicating that DEHP and DEHA produced carcinogenic effects in rats and mice (114,115). By 1984, certain European countries had either passed or were considering passing legislation relating to the level of plasticisers permitted in food contact plastics. Italy had banned DEHP and DEHA in plastics and rubbers for use in contact with fatty foods, Sweden was considering a limit of 1-2 $mgdm^{-2}$ DEHA migration from plastic packaging into cheese and the Council of Europe recommended a limit of 20 or 40 $mgkg^{-1}$ for phthalate plasticisers and 60 $mgkg^{-1}$ for adipate, tartrate, sebacate and azelate plasticisers (116).

There was, however, a certain degree of controversy about the NTP results. The European Confederation of Industrial Chemical Manufacturers sponsored research into the relevance to man of the plasticiser toxicological studies which had been carried out on rodents. This research concluded that DEHP was not a genotoxic carcinogen and that the NTP studies with rodents were unlikely to be relevant to human exposure (117).

In this climate of uncertainty in the early 1980's the UK Ministry of Agriculture, Fisheries and Food (MAFF), Working Party on Chemical Contaminants from Food Contact Materials, endeavoured to assess the

health risk that plasticiser contamination of food represented to the UK population and thus what action, if any, should be taken. At that time however, insufficient information was available on which plasticisers, and at what levels, were used in UK food contact plastics. As indicated early the majority of studies have measured the migration into food simulants and not real foods. Therefore the information required to assess the potential exposure to, and thus health risk from, plasticisers was identified and a programme of work instigated to provide this information. The work reported here forms part of that programme.

CHAPTER 2

MIGRATION OF DEHA FROM PVC CLING FILM INTO FOOD UNDER TYPICAL DOMESTIC USE CONDITIONS

2.1 INTRODUCTION

Plasticiser contamination of food may result from two discrete areas of use of plasticised packaging materials. The domestic and the retail use of plasticised packaging materials. In order to estimate the dietary intake of plasticisers the level of plasticiser contamination of food resulting from both these uses must be determined. This chapter deals with plasticiser contamination of food resulting from the domestic use of plasticised packaging materials and chapter two deals with plasticiser contamination of food due to the retail use of plasticised packaging materials.

The most commonly used plastic food contact material in the home is DEHA plasticised PVC, generally known as cling film. Research carried out in Sweden indicated that DEHA migrated readily from cling film into cheese. The uppermost slice of a block of cheese which had been in contact with cling film could contain up to 1,300 $mgkg^{-1}$ of DEHA (103). However, information on the level of DEHA migration into other food types was not available. The majority of work on the migration of plasticisers from PVC into foodstuffs reported in the literature utilised food simulants rather than real foods. Thus the determination of the level of DEHA migration from cling film into a variety of food types was required in order to assess dietary intake levels of DEHA due to the domestic use of cling film.

Domestic usage of cling film can vary dramatically between households and therefore a small survey, via a questionnaire, was carried out by MAFF to establish likely household practice, including types of food

wrapped and storage time and temperature. The results of this survey were taken into consideration during the planning of the subsequent migration studies. Three food types are considered in this work; cooked meats, cakes, and fruit and vegetables. The level of DEHA migration into other food types and food prepared according to published recipes and cooked in a microwave oven in contact with cling film is reported elsewhere (106).

The method employed to determine the level of DEHA contamination in food was a stable isotope dilution technique and used an initial solvent extraction of the food followed by size exclusion chromatography (SEC) to separate the analyte and internal standard from coextracted food lipids. Subsequent quantification was by gas chromatography-mass spectrometry (GC-MS).

2.2 EXPERIMENTAL

2.2.1 MATERIALS

The internal standard deuterated DEHA (d_4-DEHA) was available in the laboratory from earlier work (104). The primary standard DEHA (Hexaplas DOA, ICI) was commercially available. Both standards were better than 96% pure when analysed by capillary GC. All solvents were of HPLC-grade and supplied by Rathburn (Walkerburn, Scotland) unless stated otherwise.

The cling film used for this work was a 12µm thick DEHA (17.2 % w/w) plasticised PVC film (Filmco International Ltd.) and was of a grade and type typically used in the home. The level of DEHA in the film was determined by capillary GC analysis of a chloroform extract of the

film and was in agreement with the value provided by the manufacture (119). Foodstuffs were purchased from local retail outlets where it had been established that the products had not previously been in contact with plasticised film. This was confirmed by the analysis of blanks.

2.2.2 INSTRUMENTATION

2.2.2.1 Open Column Size Exclusion Chromatography (120)

Column	: 1m × 25mm I.D. glass column (Pharmacia) packed with 800 mm bed of Bio-Beads SX-3 (Bio-Rad)
Solvent	: Dichloromethane/cyclohexane 1:1 v/v, 3.0 $mLmin^{-1}$
Pump	: Model 6000A (Waters Associates)
Injector	: WISP710B auto sampler (Waters Associates) (1·5mL)
Detector	: LC-UV (Pye Unicam) (254 nm)
Chart Recorder	: Servoscribe 1s (Labdata)
Fraction Collector	: Helirac 2212 (LKB)

2.2.2.2 Selected Ion Monitoring Gas Chromatography-Mass Spectrometry

Column	: 30m × 0.2433mm I.D. fused silica column, 0·25µm bonded phase DB5 (J&W Scientific)
GC	: 4160 HRGC (Carlo Erba Strumentazione)
Carrier Gas	: Helium at 1 $mLmin^{-1}$
Injection Volume	: 1µL split ≃ 30:1
Mass Spec.	: 7070H mass spectrometer (VG) with electron impact at 70 eV and a 200 µA trap current operated at 400 resolution
Data Handling	: 11/250 VG data handling

2.2.3 MIGRATION EXPERIMENTS

Three replicate migration experiments were carried out for each food type. The experiments were conducted in an as reproducible a manner as possible, particular effort was made to ensure that the film was uniformly and reproducibly stretched when wrapping samples. The total and sub-sample weights, total amount of film used, and area of film in contact with the sample were recorded.

Slices of meat were cut from cooked joints and over-wrapped to simulate the storage of partially consumed joints of meat. Although the wrapping of warm meat after cooking is not recommended on hygiene grounds, it does occur and therefore slices of meat both at room temperature and at 50°C were over-wrapped and stored in a refrigerator. Slices of cake, as might be taken in a packed lunch, were over-wrapped with film and stored at room, refrigerator and domestic deep freeze temperature for various lengths of time. Cut fruit and vegetables were completely over-wrapped and stored at domestic refrigerator temperature to simulate storage following partial consumption. Slices of cheese were exposed to either a single or double layer of film to determine the extent to which a second layer of film would contribute to contamination of the food.

2.2.3.1 Cooked Meats

Samples of cooked sliced chicken, ham and salami (30g of each) were placed on a glass plate, over-wrapped with film and stored for five days at 5°C. A whole chicken and joints of beef, lamb and pork were cooked in the laboratory kitchen. A slice (ca. 30g) of meat was cut from the joint placed on a glass plate and over wrapped with film

either when the meat was still warm (50°C) or once the meat had cooled to room temperature. In both cases, the samples were stored for five days at 5°C.

2.2.3.2 Cakes

Small iced cakes (Lyons cup cake, chocolate flavour) and slices (ca.1 cm thick) of swiss roll (Lyons swiss roll, chocolate flavour), madeira cake (Park Cakes), fruit cake (Memory Lane Genoa cake) and battenburg cake (Littlewoods) were placed on glass plates, over-wrapped with film and stored for either one or seven days at room temperature or seven days at 5°C or -18°C.

2.2.3.3 Fresh Fruit and Vegetables

Half a cucumber, a grapefruit, an avocado with the stone removed and a small cabbage were each wrapped completely in film and stored at 5°C for five days.

2.2.3.4 Multiple Layer Experiment

One side of a glass plate (ca. 20 × 20 cm) was covered with film and a second layer added to one half of the glass plate. Four cheddar cheese slices (85 × 60 × 4 mm) were placed on the glass plate and a second glass plate which had been identically prepared placed on top. Thus two cheese slices were sandwiched between glass plates covered with one layer of film each and two cheese slices were sandwiched between glass plates covered with two layers of film each. The samples were stored for either one or seven days at 5°C.

2.2.4 ANALYSIS

2.2.4.1 Extraction

Samples were weighed accurately and homogenized (Ultra-Turrax) in acetone/hexane (1:1 v/v, 150 mL), after the addition of d_4-DEHA internal standard (0·5-3·0 mg according to food type). The homogenized mixture was set aside overnight to allow the internal standard and analyte to equilibrate. The supernatant was then decanted from the homogenate which was then extracted with two further portions of solvent (75 mL each). Distilled water (1-5 mL) was added to high sugar content samples to aid extraction. Samples with a high water content (cucumber and grapefruit) required centrifugation (Coolspin 2, MSE) to separate the aqueous and organic layers. The combined extracts were dried (Sodium sulphate, Na_2SO_4), evaporated to dryness, and the residue (largely extracted fat) re-dissolved in dichloromethane/ cyclohexane (1:1 v/v, DCM/C_6H_{12}) to give a solution of not more than 0·3 g of fat per mL of solvent. Particulate matter was removed by low-speed centrifugation (Centaur 2, MSE) prior to clean-up by SEC.

2.2.4.2 Clean-up

DEHA was separated from co-extracted fat using the automated SEC system described in section 2.2.2.1. The column effluent was monitored at 254nm and samples interspersed with solutions of phthalate esters, as uv-active retention time markers, to monitor the flow rate during unattended operation. The DEHA/d_4-DEHA containing fraction (60-66 min) was collected, evaporated to dryness under reduced pressure and transferred quantitatively to a 1·5 mL vial using acetone. The collection time had previously been established by the GC analysis of two minute fractions of the SEC eluant of a DEHA standard.

2.2.4.3 SIM GC-MS Analysis

GC-MS analysis was carried out on the system described in section 2.2.2.2. The ions at m/z = 129 (DEHA) and 133 (d_4-DEHA) were monitored and quantification was based on standard calibration curves for DEHA/d_4-DEHA at known weight ratios. Food spiked with known amounts of DEHA were analysed as positive controls.

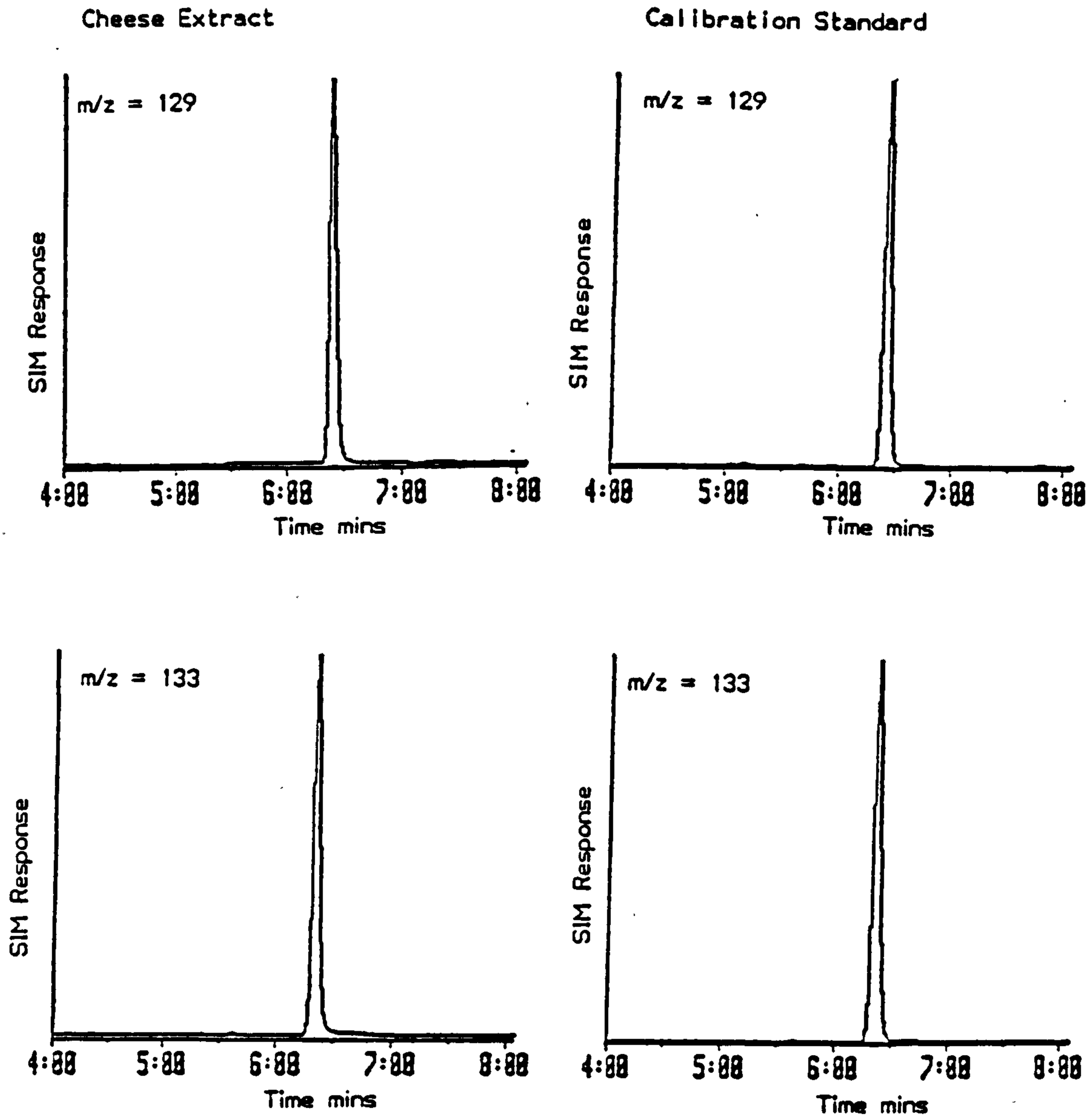
2.3 RESULTS AND DISCUSSION

2.3.1 ANALYTICAL METHODOLOGY

The method of analysis for DEHA in a variety of food types has been discussed in detail in the literature (104). The use of a stable isotope dilution technique compensates for losses during the analytical procedure and negates the need to determine (and allow for) the recovery of DEHA through the method, for each food type. It also permits partial recovery of the analyte and internal standard during the SEC clean-up stage which allows peak-cutting to avoid the tail of a partially resolved lipid peak which could cause GC column deterioration and interference in the GC-MS analysis.

The SIM GC-MS chromatograms of contaminated food samples showed smooth, clean, symmetrical peaks for both d_0-DEHA and d_4-DEHA. Some typical examples are given in Figure 1, which show that the chromatograms for the samples and standards were virtually identical. The deuterated adipate eluted from the column three to four seconds earlier than the non-labelled form. This phenomena is commonly

Figure 1. SIM GC-MS Chromatograms of a Calibration Standard and Contaminated Cheese Extract. m/z = 129 d_0-DEHA, m/z = 133 d_4-DEHA.



SIM GC-MS Conditions: 30m × 0·24mm I.D. DB5 fused silica column operated at a helium carrier gas flow rate of 1 mLmin^{-1} at an isothermal column temperature of 220°C. Ions monitored were for dwell times of 10ms to give a cycle time of 250ms.

observed in the capillary GC analysis of deuterated analysis. Calibration curves (figure 2) were linear and reagent and sample blanks analysed satisfactorily. The relative standard deviation (RSD) for the method was 1% based on replicate analysis of sub-samples of cheese contaminated at 5·4 $mgkg^{-1}$ (104).

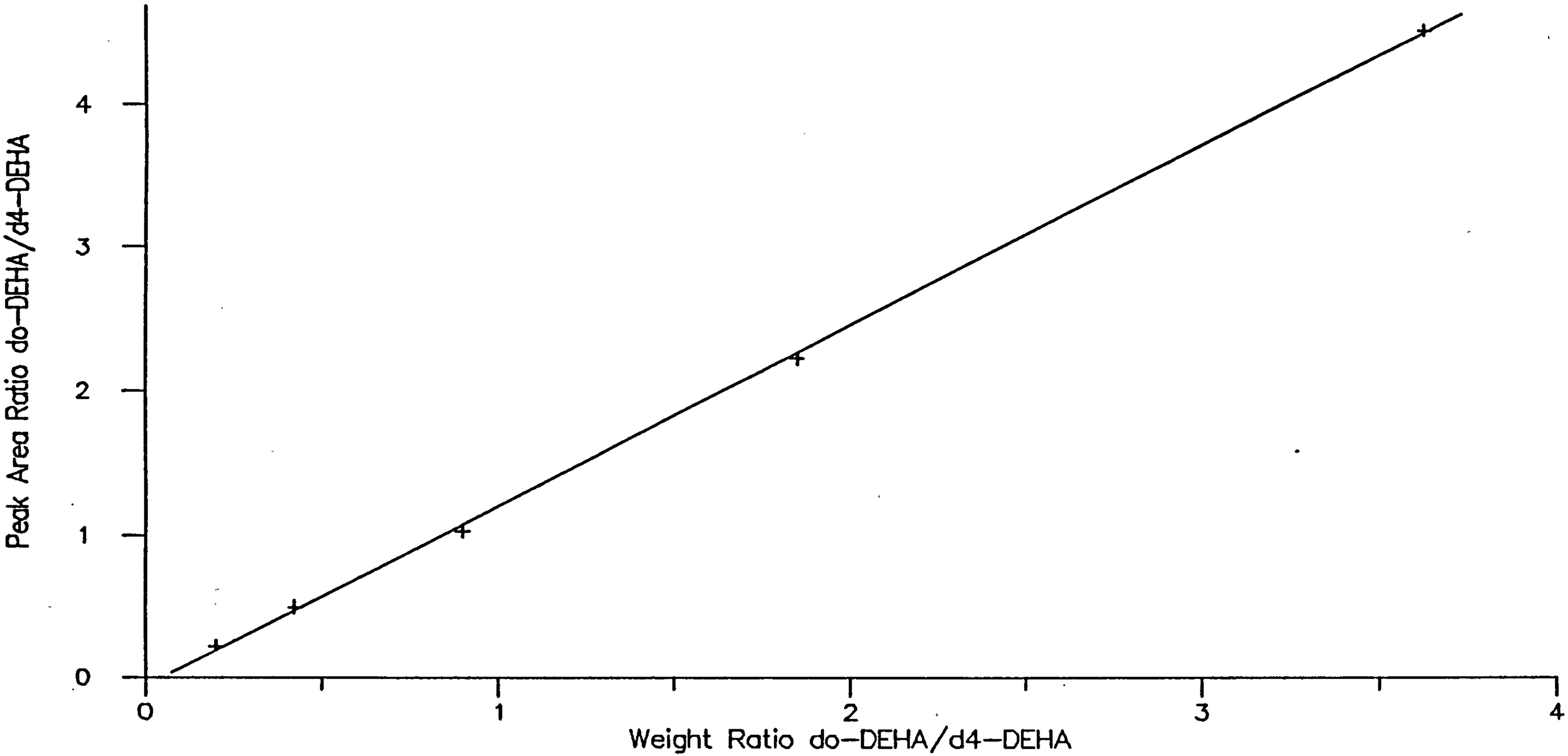
2.3.2 MIGRATION DATA

2.3.2.1 General Points

The reproducibility of the migration experiments was generally poor. For example, the level of DEHA found in the cake samples (table 4), where one might expect a reproducible level of DEHA migration into similar slices of a single cake, had a RSD of between 15% to 65% for triplicate samples. The poor reproducibility of migration experiments has been assigned to a combination of factors. These include, the difficulty of reproducibly stretching the film when wrapping; variations in contact area; inaccuracies in determining the contact area (a certain degree of estimation was required to determine the contact area for a slice of meat, or half an avocado, for example) and the heterogeneous nature of the surface fat of some of the samples. Even though the reproducibility of the migration experiments was poor, some trends in the migration levels are discernible.

The results are expressed in both $mgkg^{-1}$ for dietary intake calculation purposes and $mgdm^{-2}$ to put the level of migration in context with the impending EC limit of 10 $mgdm^{-2}$ overall migration from food contact plastics. Expression of the results as loss of plasticiser from the film ($mgdm^{-2}$) allows the calculation of the migration level into samples of food of different sizes to those

Figure 2. Calibration Graph for the Isotope Dilution GC–MS Analysis of DEHA. Plot of Peak Area Ratio Versus Weight Ratio of do–DEHA/d4–DEHA.



Slope = 1.25
Correlation Coefficient =0.9998
Intercept (x = 0) = –0.06

considered in this work. However, care must be taken when using the results expressed in $mgdm^{-2}$ as, for some food types, it was difficult to determine the contact area precisely. Another factor that should be taken into account when calculating migration levels, is whether or not any over-wrapping of film has occurred. The results of experiments using either a single or double layer of film for wrapping (table 1) indicate that a second layer of film contributes to the overall level of migration. The mean increase due to an additional layer of film when cheese was stored at 5°C for one day was 8·7 $mgdm^{-2}$ (57 %) and 9·5 $mgdm^{-2}$ (50 %) for five days storage. Thus, if the level of migration into a whole cake, for example, was to be calculated, some allowance for the over-wrapping would have to be made.

Table 1. Migration of DEHA into Cheddar Cheese in Contact with One or Two Layers of Film.

	Exposure and migration	
Sample	1 day at 5°C	7 days at 5°C
	$mgdm^{-2}$	$mgdm^{-2}$
Single Layer	15·3	18·4
	15·0	19·7
Double Layer	24·8	27·4
	24·5	28·1

2.3.2.2 Migration into Sliced Processed Meat and Cooked Meats

The levels of DEHA migration into sliced processed meat and cooked meats are given in tables 2 and 3 respectively, and range from 3 mg kg^{-1} (0·3 $mgdm^{-2}$) to 200 $mgkg^{-1}$ (16 $mgdm^{-2}$). There appears to be a relationship between the fat content of the meat and the level of DEHA migration. In all cases the maximum migration observed was for the

meat which had the highest fat content. For example, the mean migration level into salami, (45 % fat (121)) exposed for seven days at 23°C was 180 $mgkg^{-1}$ (14 $mgdm^{-2}$) compared with 75 $mgkg^{-1}$ (5 $mgdm^{-2}$) into chicken (5% fat (121)) under identical conditions. However, the relationship between fat content and level of DEHA migration does not appear to be a simple one. The mean migration into lamb (18% fat (121)), when covered cold and stored for seven days at 5°C, was 12 $mgkg^{-1}$ (1·2 $mgdm^{-2}$) whereas the mean migration into pork (20% fat (121)) was 50 $mgkg^{-1}$ (5 $mgdm^{-2}$) under identical conditions. This represents a 320 % increase in migration for a 11% increase in fat content. However, if one considers the levels of migration observed into chicken (5% fat (121)) and salami (45% (121)) when exposed to film for seven days at 23°C (table 2) the increase in the level of migration is only 140% even though the fat content of salami is 800% higher than that of chicken. The reason for this apparent discrepancy may be differences in the fraction of the fat present at the surface. Fat in the salami is dispersed throughout the food whereas chicken has a layer of fat at the surface.

Table 2. Migration of DEHA into Sliced Processed Meat Stored for Seven days at 23°C or Thirty Days at -18°C.

Food Type (% Fat)	Exposure and Migration			
	7 Days at 23°C		30 day at -18°C	
	$mgkg^{-1}$	$mgdm^{-2}$	$mgkg^{-1}$	$mgdm^{-2}$
Chicken (5% †)	78	5·8	30	2·6
	77	5·6	31	2·4
	78	4·7	30	2·9
Mean	75	5·4	29	2·6
Ham (5%)	160	4·1	30	0·8
	89	2·6	18	0·6
	71	1·7	28	0·9
Mean	107	2·8	25	0·8
Salami (45%)	170	10·3	113	9·4
	172	14·6	114	9·4
	200	15·6	101	9·1
Mean	181	13·5	109	9·3

† Whole roast chicken, meat only
Fat content taken from reference 121

Table 3. Migration of DEHA into Cooked Meat Stored for Seven Days at 5°C.

Food Type	Migration and Covering Conditions			
	Covered Cold		Covered at 50°C	
(% Fat)	$mgkg^{-1}$	$mgdm^{-2}$	$mgkg^{-1}$	$mgdm^{-2}$
Chicken	43	3·0	94	4·4
(14% †)	38	3·7	67	4·3
	57	2·8	85	6·4
Mean	46	3·2	82	5·0
Lamb	21	2·3	39	3·5
(18% ‡)	7	0·5	61	7·1
	9	0·8	67	10·4
Mean	12	1·2	56	7·3
Beef	6	0·6	6	0·6
(11% ◊)	3	0·3	12	1·0
	14	0·7	25	2·1
Mean	8	0·9	14	1·2
Pork	42	5·8	68	5·6
(20% *)	54	4·9	105	10·8
	54	5·1	91	8·5
Mean	50	5·3	88	8·3

† Whole roast chicken meat & skin
‡ Roast leg of lamb
◊ Roast topside
* Roast leg of pork
Fat content taken from reference 121

The level of migration into slices of cooked meat was higher when the meat was at 50°C when covered than when it was at room temperature when covered. The high level of migration into slices of cooked chicken may be due to the high fat content of the skin which was not removed from the sample and was in direct contact with the film.

2.3.2.3 Migration into Cake, Fruit and Vegetables

The level of DEHA migration into cakes, table 4, ranged from 0·3 $mgkg^{-1}$ (0·1 $mgdm^{-2}$) to 177 $mgkg^{-1}$ (10 $mgdm^{-2}$) and would also appear to reflect the importance of fat form and content. The very high migration levels into swiss roll may be due not only to the fat content of the sponge, but also to the high fat filling which was in direct contact with the film. The importance of temperature is also apparent if one considers the results obtained for the samples' exposure for seven days at either 23°C, 5°C or -18°C. In all cases the mean migration value increases with temperature.

The migration of DEHA into the fruit and vegetables (table 5) was negligible with the exception of that into avocado which had a mean value of 53 $mgkg^{-1}$ (11 $mgdm^{-2}$). This can be attributed to the relatively high fat content of avocados, typically 22% by mass.

Table 4. Migration of DEHA into Cakes Stored for One Day at 23°C or Seven Days at 23°C, 5°C or -18°C.

Food Type (% Fat)	Exposure and Migration							
	7 Days @23°C		1 day @ 23°C		7 days @ 5°C		7 days @ -18°C	
	$mgkg^{-1}$	$mgdm^{-2}$	$mgkg^{-1}$	$mgdm^{-2}$	$mgkg^{-1}$	$mgdm^{-2}$	$mgkg^{-1}$	$mgdm^{-2}$
Chocolate	6	0·6	2	0·2	1	0·1	0·3	<0·1
Cup Cake	6	0·6	9	1·0	5	0·5	0·3	<0·1
	13	1·4	5	0·6	6	0·7	0·4	<0·1
Mean	13	0·9	5	0·6	4	0·4	0·3	<0·1
Swiss Roll	207	13·2	42	3·3	104	7·6	46	3·0
(27% †)	150	8·3	73	4·4	102	6·9	80	4·8
	174	9·6	131	7·6	170	11·7	63	4·3
Mean	177	10·4	82	5·1	125	8·7	63	4·0
Madeira	90	4·5	14	0·9	47	2·7	2	0·1
(17%)	56	2·7	19	1·0	16	0·9	2	0·1
	84	3·8	9	0·5	40	2·4	13	0·8
Mean	77	3·7	14	0·8	34	2·0	6	0·3
Battenburg	22	1·5	4	0·4	7	0·6	0·3	<0·1
	17	1·2	2	0·2	6	0·5	3	0·3
	22	1·5	3	0·3	4	0·4	4	0·4
Mean	20	1·4	3	0·3	6	0·5	2	0·3
Fruit Cake	44	3·2	7	0·6	8	0·6	2	0·3
(11%)	22	3·2	7	0·6			4	0·3
	40	2·7	13	1·1	7	0·7	1	0·1
Mean	35	2·4	9	0·8	7·5	0·6	2	0·2

† Sponge cake made with fat
Fat content taken from reference 121

Table 5. Migration of DEHA into Fresh Fruit and Vegetables Stored for Five Days at 5°C.

Food Type (% Fat)	Migration mgkg^{-1}	mgdm^{-2}	Food Type (% Fat)	Migration mgkg^{-1}	mgdm^{-2}
Avocado (22%)	43	10·3	Cabbage (Tr.)	5	0·3
	59	11·7		9	0·4
	57	11·2		0·5	<0·1
Mean	53	11·1	Mean	4·8	0·3
Cucumber (0·1%)	1	<0·1	Grapefruit (Tr.)	3	0·3
	0·3	<0·1		1	<0·1
	0·3	<0·1		5	0·6
Mean	0·5	<0·1	Mean	3	0·3

Tr. Trace levels of fat
Fat content taken from reference 121

In summary, the level of DEHA migration from PVC cling film into food under simulated domestic use varies greatly and can be up to 200mgkg^{-1} (salami exposed for seven days at 23°C, table 2). The variation in the level of DEHA migration is due to a number of factors including; the fat form, whether the fat is concentrated at the surface or dispersed throughout the food, and content of the food, the length and temperature of exposure and the manner in which the food is wrapped, that is the extent the to which the film is stretched and whether there is over-wrapping of the film.

CHAPTER 3

PLASTICISER CONTAMINATION OF FOOD RESULTING FROM THE RETAIL USE OF FLEXIBLE FILMS

3.1 INTRODUCTION

On completion of the work to determine the level of plasticiser contamination of food due to domestic usage of PVC cling film (see chapter two) attention was focussed on contamination levels due to the retail use of plasticised flexible films. Previous work had indicated that it is difficult to obtain reproducible migration data using real foods. It was therefore decided not to analyse large numbers of each food type (as appreciable variation in the level of migration was inevitable) but to survey a diverse range of food types and outlets thereby surveying the average level of the contamination.

To this end, 156 samples from four major national supermarket chains and a number of smaller outlets such as snack-bars, cafeterias, sandwich shops and a petrol filling station, were analysed. As for the simulated domestic use of PVC cling film, analysis involved solvent extraction, SEC clean-up and end determination by either GC-MS or GC with flame ionization detection (GC-FID).

Three types of plastic films were found to be in retail use, DEHA plasticised PVC, polyvinylidene chloride (PVDC) plasticised with either dibutyl sebacate (DBS) or acetyl tributyl citrate (ATBC) and nitrocellulose-coated regenerated cellulose film (RCF) plasticised with two or more phthalate ester plasticisers.

Cooked and uncooked meat and poultry was exclusively wrapped in DEHA plasticised PVC "meat wrap" cling film which was also used to a much lesser extent in the packaging of cheese, fruit and vegetables and

baked goods. DBS plasticised PVDC was used to over-wrap blocks of cheese or for 'chub packs' (a sausage-type casing) of smoked cheese and meat products while ATBC plasticised PVDC was exclusively used in the packaging of cheese. Phthalate plasticised RCF was extensively used in the packaging of meat pies and baked goods.

3.2 EXPERIMENTAL

3.2.1 MATERIALS

3.2.1.1 Primary and Internal Standards and Solvents

The following primary standards were available commercially; ATBC (Croda Chemicals), butylbenzyl phthalate (BBP, Santicizer 160, BP Chemicals), dibutyl phthalate (DBP, Bisoflex, BP Chemicals), DBS (Sigma Chemical Company), dicyclohexyl phthalate (DCHP, British Cellophane Ltd.), DEHA (Hexaplas DOA, ICI), di(2-ethylhexyl) phthalate (DEHP, Genomoll 100, Hoechst), diethyl phthalate (DEP, Bisoflex, BP), diphenyl 2-ethylhexyl phosphate (DPOP, Santiciser 141, B&T Polymers). All standards were better than 96% pure when analysed by capillary GC.

The following internal standards were available in the laboratory; deuterated DEHA (d_4-DEHA), deuterated DBP (d_4-DBP), deuterated DCHP (d_4-DCHP) and deuterated DEP (d_4-DEP) (104,101) and diheptyl phthalate (DHP, Polyscience) was commercially available.

All solvents were of HPLC-grade and supplied by Rathburn (Walkerburn, Scotland) unless stated otherwise.

3.2.1.2 Foodstuffs

Foodstuffs were purchased from four major national supermarket chains and a number of smaller outlets such as snack-bars, cafeterias, sandwich shops and a petrol filling station in Norwich. The samples were stored in their original packaging under normal conditions (i.e. refrigerated or at room temperature) until their "sell-by" or "best-by" date. Fresh meat samples were kept for a further two to three days past their "sell-by" date to allow for further storage that might occur prior to food preparation. Items that had no date stamp or that had a very long shelf life, confectionery for example, were kept typically for two weeks. During this storage period, a chloroform extract of a portion of the packaging film was analysed by GC to establish the presence, identity and level of plasticisers. At the end of the storage period details of the food and its packaging were recorded including its weight, the food-film contact area and the use of any additional packaging materials such as card inserts or aluminium trays. The food was then unwrapped and analysed for the plasticisers found to be present in the packaging. Blank samples were obtained from local retail outlets where it had been established that the product had not previously been in contact with plasticised materials. An alternative source of blanks was core samples taken from the survey samples remote from the food packaging contact layer.

3.2.2 INSTRUMENTATION

3.2.2.1 Gas Chromatography

Column	: 25m × 0.23mm I.D. fused silica column, 0·12µm bonded phase CP Sil-5 CB (Chrompack)
GC	: 4160 HRGC (Carlo Erba Strumentazione)
Carrier Gas	: Hydrogen at 3 mLmin^{-1}
Auto Injector	: AS 570V (Carlo Erba Strumentazione)
Injection Volume	: 1µL split ≃ 30:1
Detector	: Flame ionisation
Data Handling	: Trilab 2000 (Trivector)

3.2.3 ANALYSIS

3.2.3.1 Analysis of Packaging Material

A portion of the film (20 mg) remote from the food was taken and infused overnight in chloroform (4 mL) containing DEP and DCHP (0.1 mgmL^{-1} of each) as GC internal standards. The sample was then shaken (Orbital shaker, Luckham Ltd) for one hour and the chloroform extract analysed directly using the GC system described in section 3.2.2.1 and the following column temperature programme; 140°C for 2 min, 20°Cmin^{-1} to 225°C and hold 3 min, 20°Cmin^{-1} to 310°C and hold 5 min. Identification was based on retention time and quantification on peak area.

3.2.3.2 Analysis of Food Samples

Phthalates are ubiquitous in the environment. Therefore, to achieve low phthalate backgrounds, all glassware was rinsed with "glass-distilled" acetone immediately prior to use and only "glass-distilled" or "HPLC-grade" solvents were used. Similarly, contact of food or food

extracts with materials other that glass, PTFE, stainless steel, or their original packaging, was avoided.

Extraction

The sample was unwrapped and, where appropriate, boned and re-weighed. Samples greater than 50g were homogenized in a domestic food processor (Type 4243, Braun) and an accurately weighed sub-sample (ca. 30g) taken for analysis. If a sample resisted such homogenization, cheese for example, all the outer surfaces were removed to a depth of approximately 5 mm and this material weighed and analysed. Products with a high sugar content, confectionery for example, were dissolved in the minimum of hot water prior to extraction. Multiple sub-samples of selected food items were taken to monitor the thoroughness of the homogenisation procedure and the reproducibility of the method.

The sub-samples were homogenized (Ultra-Turrax) in acetone/hexane (1:1 v/v, 150 mL) after the addition of a suitable internal standard(s). The choice and level of internal standard employed was based on the plasticisers identified in the packaging and was as follows: contaminant (internal standard, level); ATBC (DCHP or DEHP, 1-3 mg), BBP and DPOP (d_4-DBP and d_4-DCHP, 50-200 µg each), DBS (DHP, 1-3 mg), DBP (d_4-DBP, 50-200 µg), DCHP (d_4-DCHP, 50-200 µg), and DEHA (d_4-DEHA, 0.5-3 mg). The sub-samples were then extracted using a procedure identical to that described in section 2.2.4.1.

Clean Up

Sample clean up was as described in section 2.2.4.2. Standards were run to establish the elution times of the phthalate plasticisers (uv

detection) and DEHA, ATBC, DBS and DPOP (GC analysis of fractions). These elution times were then used to set the collection windows for quantitative collection of the SEC eluant containing the required analyte(s) and internal standard(s). To ensure the correctness of the collection windows used, mixtures of standard plasticisers in known ratios were analysed by GC before and after SEC. The SEC fractions collected for food samples were evaporated to dryness under reduced pressure and transferred quantitatively to a 1·5 mL vial using acetone.

SIM GC-MS Analysis for DEHA, BBP, DBP, DCHP and DPOP

The analysis for DEHA was identical to that given in section 2.2.4.3. Samples for BBP, DBP, DCHP and DPOP analysis were analysed on the SIM GC-MS system described in section 2.2.2.2 with the following column temperature programme, 140°C hold for 1 min 20°Cmin^{-1} to 300°C. The mass spectrometer was operated in a three-channel selected ion monitoring mode with the source held at 200°C. The ions monitored were m/z = 149 (d_o-phthalates), 153 (d_4-phthalates) and 251 (DPOP) with dwell times of 150ms per mass and settling times of 20ms to give a cycle time of 510ms. Quantification was based on ion peak areas (intensity versus time), judged against calibration curves constructed for standard mixtures of analyte and d_4-internal standards at various weight ratios.

GC Analysis for ATBC and DBS

Samples for ATBC and DBS analysis were analysed by GC-FID using the system outlined in section 3.2.2.1 and the following temperature programmes: ATBC (with DCHP internal standard), 215°C hold 6 min,

20°Cmin-1 to 300°C and hold 5 min; ATBC (with DEHP internal standard), 200°C hold 4min, 20°min-1 to 230°C hold 2min, 20°min-1 to 300°C and hold 5 min; DBS, 180°C for 7 min, 20°min-1 to 300°C and hold 5 min. The identity of the analyte was confirmed in selected samples by mass spectrometry.

3.3 RESULTS AND DISCUSSION

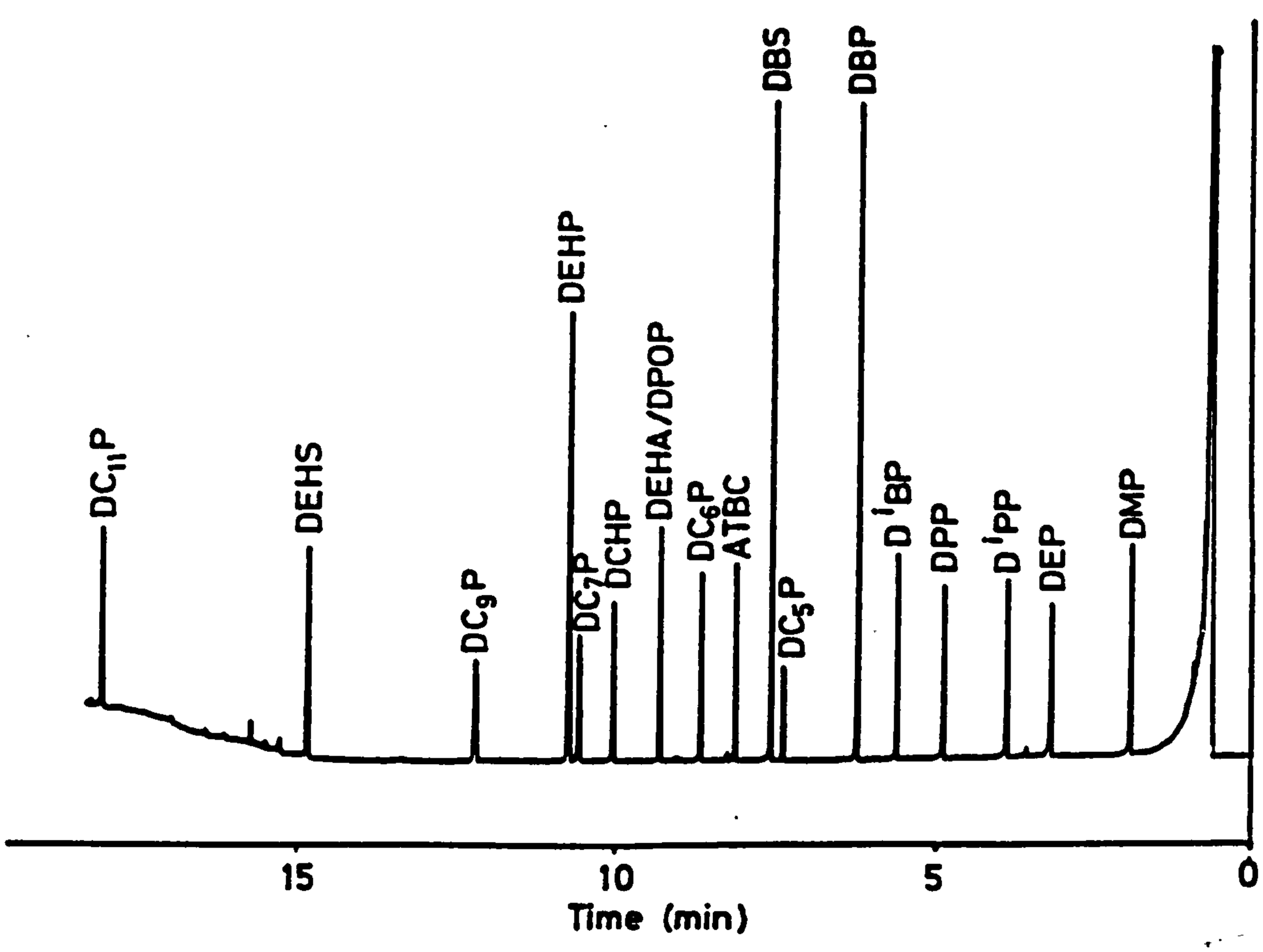
3.3.1 ANALYTICAL METHODOLOGY

3.3.1.1 Film Analysis

Extraction of plasticisers by infusion in chloroform is both rapid and quantitative for thin films when there is a high solvent to polymer ratio. This method was therefore chosen in preference to the more complex procedure of soxhlet extraction. The GC-FID analysis of the chloroform extract of the packaging material permitted a wide range of commonly used plasticisers to be identified, as illustrated by figure 3. However, two major plasticisers, DEHA and DPOP, could not be distinguished using the method outlined in section 3.2.3.1. as they co-eluted from the GC column. GC-MS analysis was used to identify these plasticisers.

DCHP and DEP were added to the extraction solution as internal standards and their peak area ratio monitored to ensure that neither plasticiser was present in the packaging material under analysis. DCHP

Figure 3. Gas Chromatographic Separation of Standard Mixture of Plasticisers.



GC conditions: 25m × 0·23mm I.D. CP SIL 5CB fused-silica column operated using hydrogen carrier gas at 3 mLmin^{-1} with FID. Temperature programming as follows; 140°C hold 2 min, 20°Cmin^{-1} to 225°C hold 3 min, 20°Cmin^{-1} to 310°C hold 5 min.

DMP - dimethyl phthalate; DEP - diethyl phthalate; DIPP - di-isopropyl phthalate; DiBP - di-isobutyl phthalate; DBP - dibutyl phthalate; DC_5P - dipentyl phthalate; DBS - dibutyl sebacate; ATBC - acetyl tributyl citrate; DC_6P - dihexyl phthalate; DEHA - di(2-ethlhexyl) adipate; DPOP - diphenyl 2-ethylhexyl phosphate; DCHP - dicyclohexyl phthalate; DC_7P - diphenyl phthalate; DEHP - di(2-ethylhexyl) phthalate; DC_9P - dinonyl phthalate; DEHS - di(2-ethylhexyl) sebacate; $DC_{11}P$ - di-undecyl phthalate.

was the preferred internal standard as it was similar in nature to the plasticisers under study and so was used for all quantification except where it was present in some of the nitrocellulose-coated RCF films. In these cases, DEP was used as the internal standard as it was not present in any of the films studied.

The identity and quantity of plasticisers found in the packaging (percentage weight) is given in tables 6-13. These results were used to indicate the identity of plasticisers likely to be present in the retail food samples. A selection of the films was examined by transmission IR spectroscopy to identify the polymer. Those plasticised with DEHA were PVC, those with ATBC or DBS were PVDC, while those with DBP and DCHP or with a cocktail of DBP, DCHP, BBP and DPOP were found to be nitrocellulose-coated RCF.

3.3.1.2 Choice of Internal Standards for Food Analysis

The internal standard of choice was the deuterated analogue of the analyte as this permitted the use of a stable isotope dilution technique, the advantages of which have been discussed in section 2.3.1. Where synthesis of deuterated analogues was not straight forward - due to the plasticiser being unsymmetrical (BBP) or the deuterated precursors not being readily available (DBS, ATBC and DPOP)- non-deuterated internal standards had to suffice. Since BBP and DPOP were always found in combination with DBP and DCHP, the d_4-analogues of the latter pair were used as the internal standards in a single GC-MS analysis for all six compounds. DHP was used as a standard for DBS and DCHP or DEHP for ATBC, with final analysis by GC-FID.

3.3.1.3 GC-MS Analysis of Food Extracts

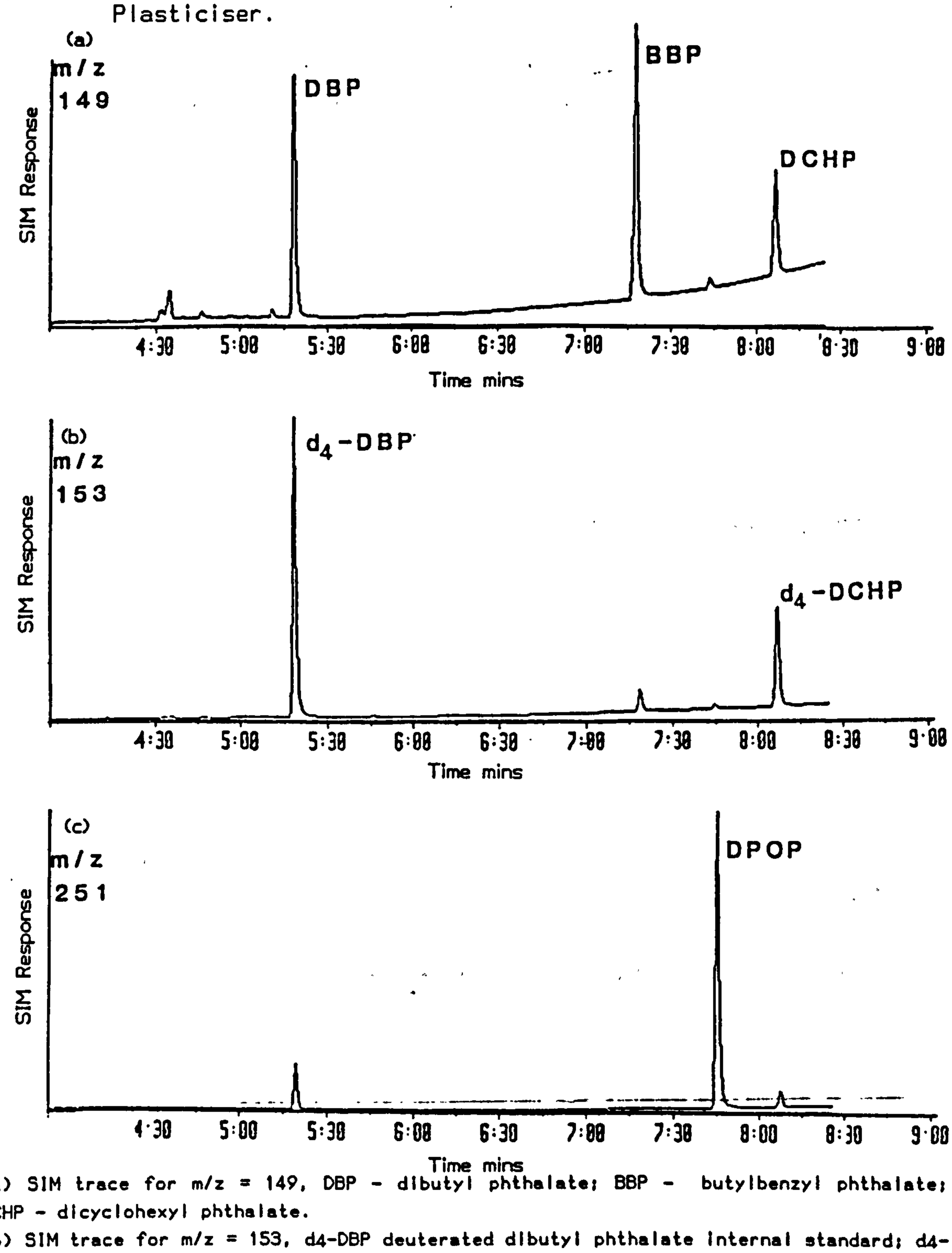
In all cases the SIM GC-MS traces showed smooth, well shaped peaks with the deuterated internal standard eluting fractionally earlier (as seen in figure 4) for both samples and standards. A typical trace for a six-component calibration standard solution is shown in figure 4. The quantitative analysis of DEHA by GC-MS has been discussed in section 2.3.1. Once again, the precision and accuracy of the method was excellent, with RSDs of 2% typically.

A typical trace for a sample wrapped in film plasticised by DBP and DCHP is given in figure 5. The figure shows the good signal to noise ratio of both the analyte and internal standard peaks and the absence of interfering peaks. The level of migration into this sample was around 15 $mgkg^{-1}$. Figure 6 shows a trace obtained for a sample that had been wrapped in film containing BBP, DBP, DCHP and DPOP. Each plasticiser was found in the food at around 6-12 $mgkg^{-1}$. Calibration curves (peak area ratio versus weight ratio) for DBP, DCHP, BBP or DPOP versus d_4-DBP or d_4-DCHP were linear at concentration ratios in the range 1:4 to 4:1 for concentrations around 5-20 μgmL^{-1}.

3.3.1.4 GC-FID Analysis of Food Extracts

The GC-FID traces obtained in the analysis for ATBC and DBS were interference-free (figure 7). The traces of blank core samples taken remote from the film food contact layer did not contain peaks at the retention time of ATBC or DBS (figure 7) thus demonstrating that the source of plasticisers was the packaging.

Figure 4. SIM GC-MS Chromatograms of Standards of Phthalate Ester Plasticiser.



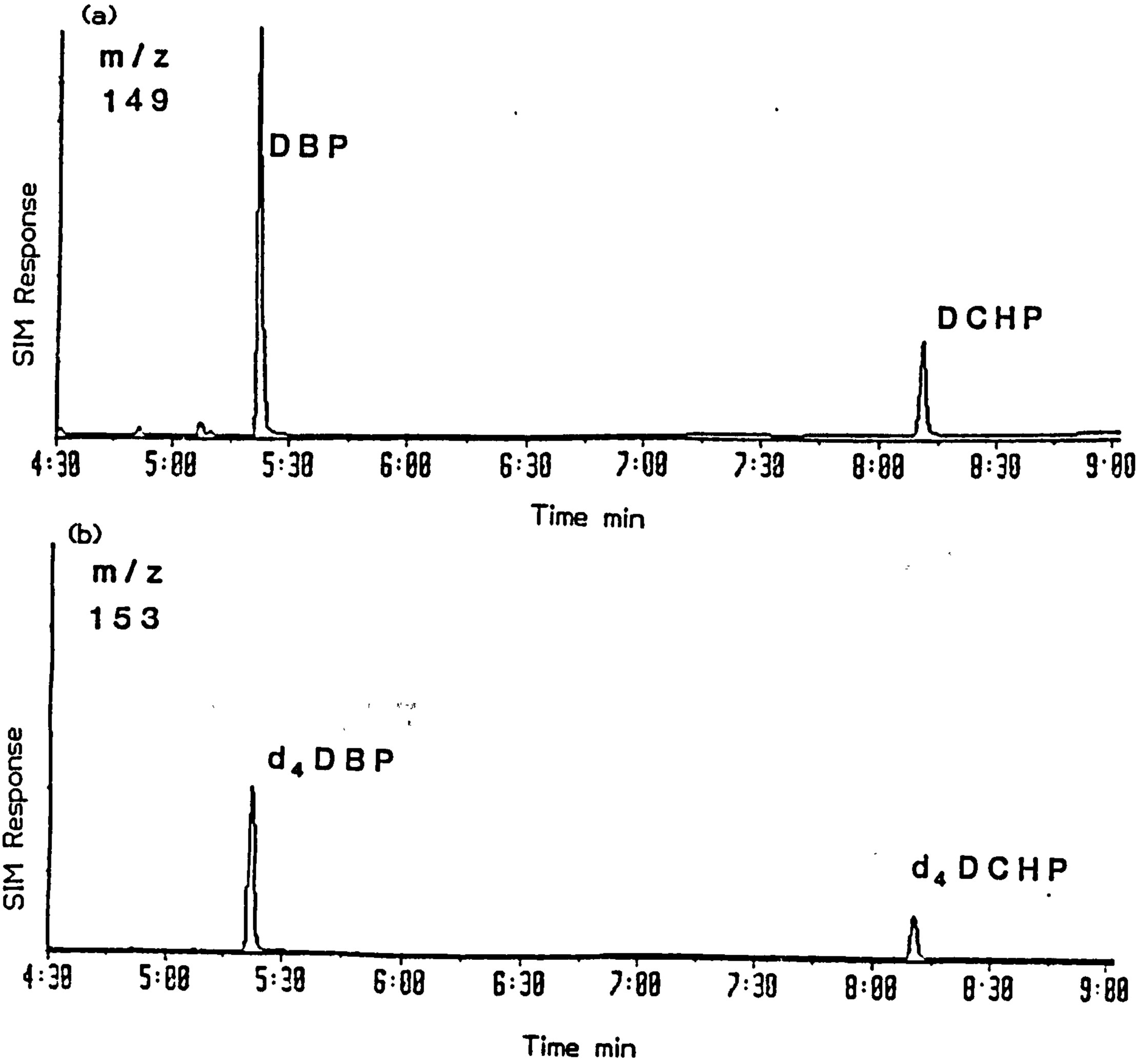
(a) SIM trace for m/z = 149, DBP - dibutyl phthalate; BBP - butylbenzyl phthalate; DCHP - dicyclohexyl phthalate.

(b) SIM trace for m/z = 153, d4-DBP deuterated dibutyl phthalate internal standard; d4-DCHP deuterated dicyclohexyl phthalate internal standard.

(c) SIM trace m/z = 251, DPOP - diphenyl 2-ethylhexyl phosphate.

SIM GC-MS conditions: 25m × 0·23mm I.D. CP SIL 5CB fused silica column operated at a helium carrier gas flow of 1 $mLmin^{-1}$ with temperature programming from 140°C at 20°$Cmin^{-1}$ to 300°C. The ions monitored were m/z = 149 d0-phthlates, 153 d4-phthlates and 251 DPOP with dwell times of 150ms and setting times of 20ms to give a cycle time of 510 ms.

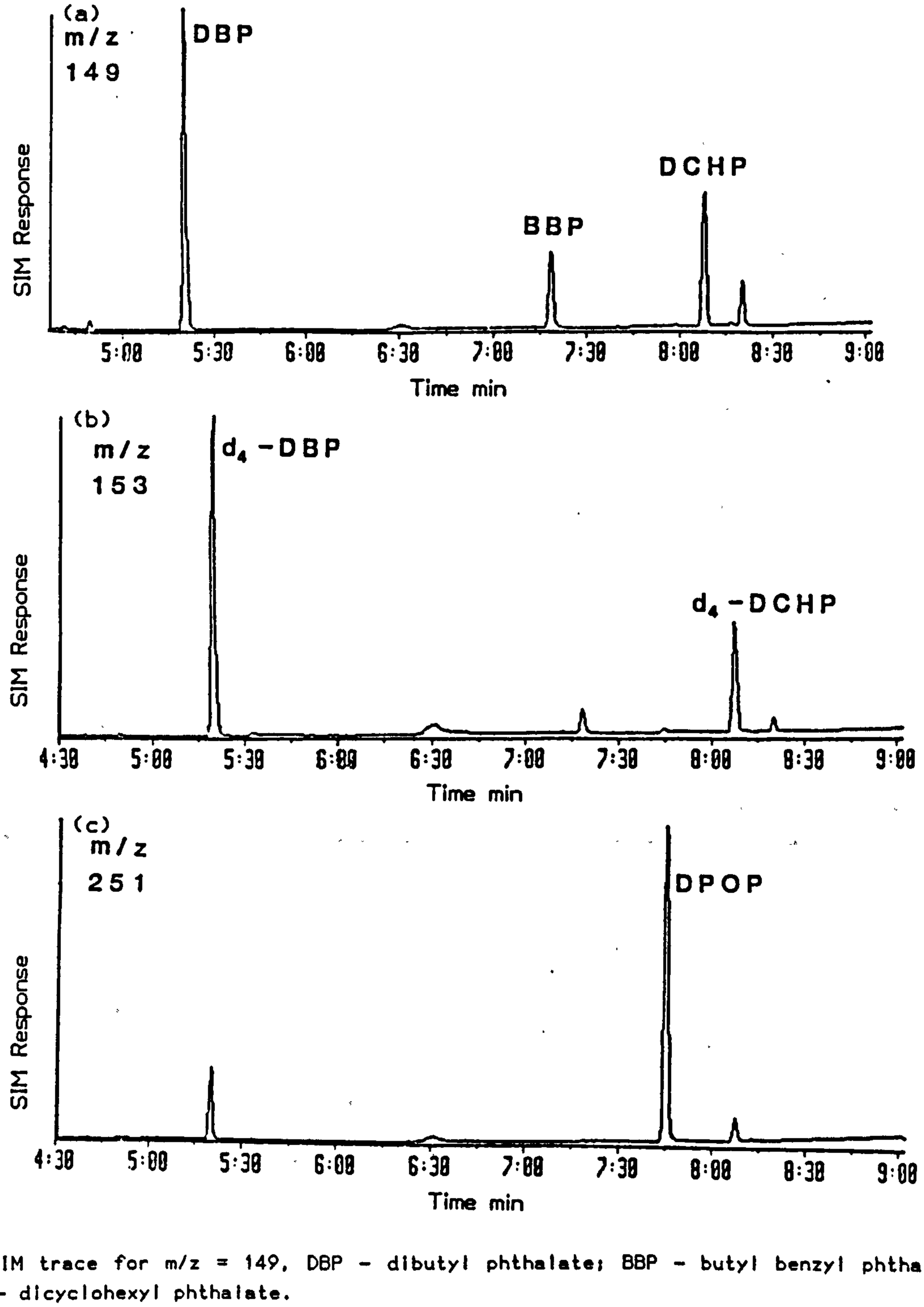
Figure 5. SIM GC-MS Chromatogram of Food Sample Contaminated at approximately 15 mgkg^{-1} level with DBP and DCHP and a Standard of d_4-DBP and d_4-DCHP.



(a) SIM trace for m/z = 149, DBP - dibutyl phthalate; DCHP - dicyclohexyl phthalate.
(b) SIM trace for m/z = 153, d_4-DBP deuterated dibutyl phthalate internal standard; d_4-DCHP deuterated dicyclohexyl phthalate internal standard.

SIM GC-MS conditions: 25m × 0·23mm I.D. CP SIL 5CB fused silica column operated at a helium carrier gas flow of 1 mLmin^{-1} with temperature programming from 140°C at 20°Cmin^{-1} to 300°C. The ions monitored with dwell times of 150ms and setting times of 20ms to give a cycle time of 510 ms.

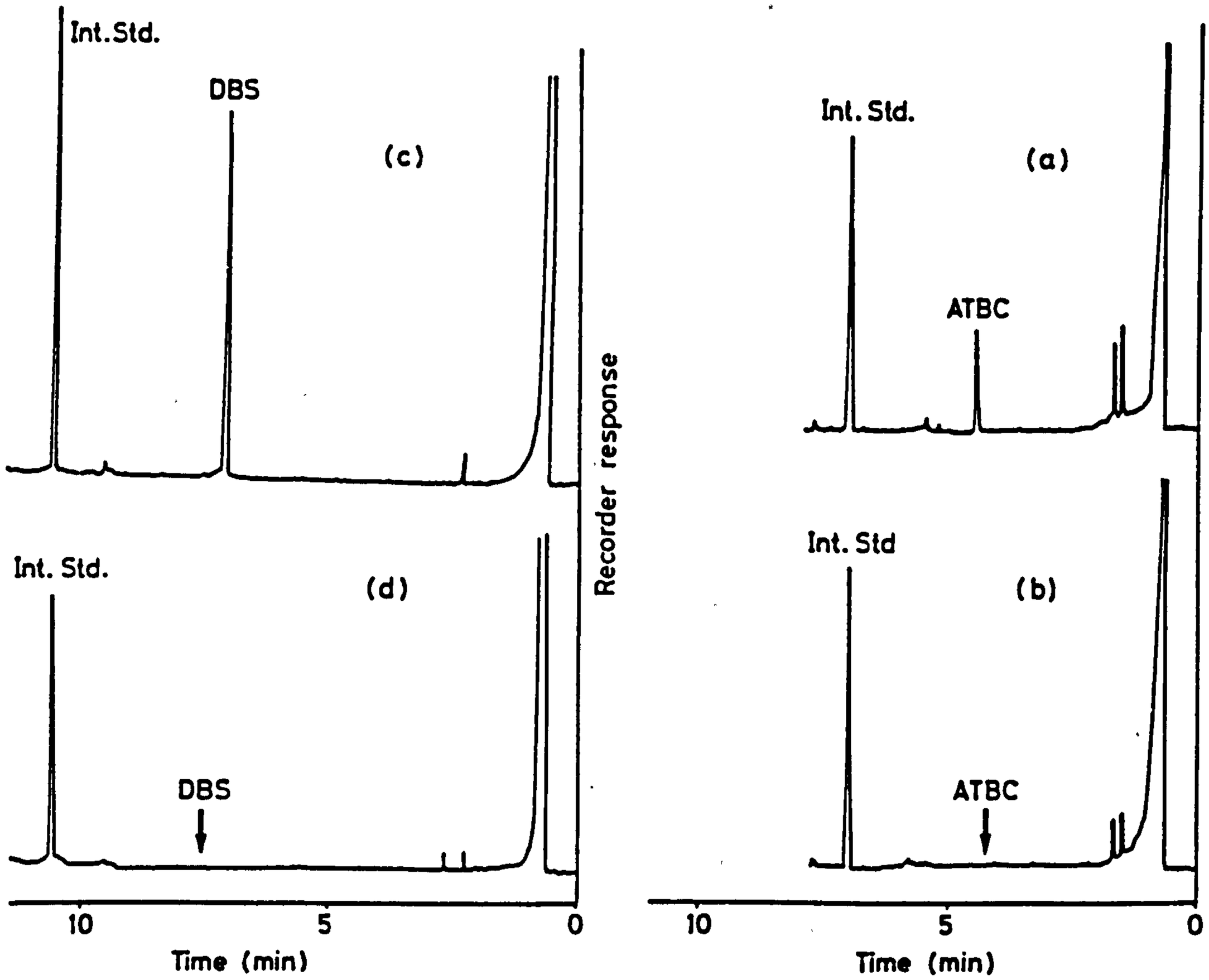
Figure 6. SIM GC-MS Chromatograms for the Analysis of DBP, BBP, DCHP and DPOP in a Pork Pie at between 6-12 mgkg^{-1}.



(a) SIM trace for m/z = 149, DBP - dibutyl phthalate; BBP - butyl benzyl phthalate; DCHP - dicyclohexyl phthalate.
(b) SIM trace for m/z = 153, d4-DBP deuterated dibutyl phthalate internal standard; d4-DCHP deuterated dicyclohexyl phthalate internal standard.
(c) SIM trace for m/z = 251, DPOP - diphenyl 2-ethylhexyl phosphate

SIM GC-MS conditions: 25m × 0·23mm I.D. CP SIL 5CB fused silica column operated at a helium carrier gas flow of 1 mLmin^{-1} with temperature programming from 140°C at 20°Cmin^{-1} to 300°C. The ions monitored with dwell times of 150ms and setting times of 20ms to give a cycle time of 510 ms.

Figure 7. Gas Chromatograms Illustrating Determination of DBS and ATBC in Food.



(a) Sample of processed cheese containing 3·2 mgkg^{-1} acetyl tributyl citrate (ATBC).
(b) Blank sample (remote from packaging contact) containing <0·05 mgkg^{-1} ATBC. Internal standard dicyclohexyl phthalate.

GC conditions: 25m × 0·23mm I.D. CP SIL 5CB fused silica column operated at 3 mLmin^{-1} hydrogen carrier gas flow rate using an FID. Temperature programming as follows : 215°C hold 6 min, 20°Cmin^{-1} to 300°C hold 5 min.

(c) Sample of smoked cheese containing 101 mgkg^{-1} dibutyl sebacate (DBS).
(d) Blank sample (remote from packaging contact) containing <0·05 mgkg^{-1} DBS. Internal standard diheptyl phthalate.

GC conditions: 25m × 0·23mm I.D. CP SIL 5CB fused silica column operated at 3 mLmin^{-1} hydrogen carrier gas flow rate using an FID. Temperature programming as follows : 180°C hold 7 min, 20°Cmin^{-1} to 300°C hold 5 min.

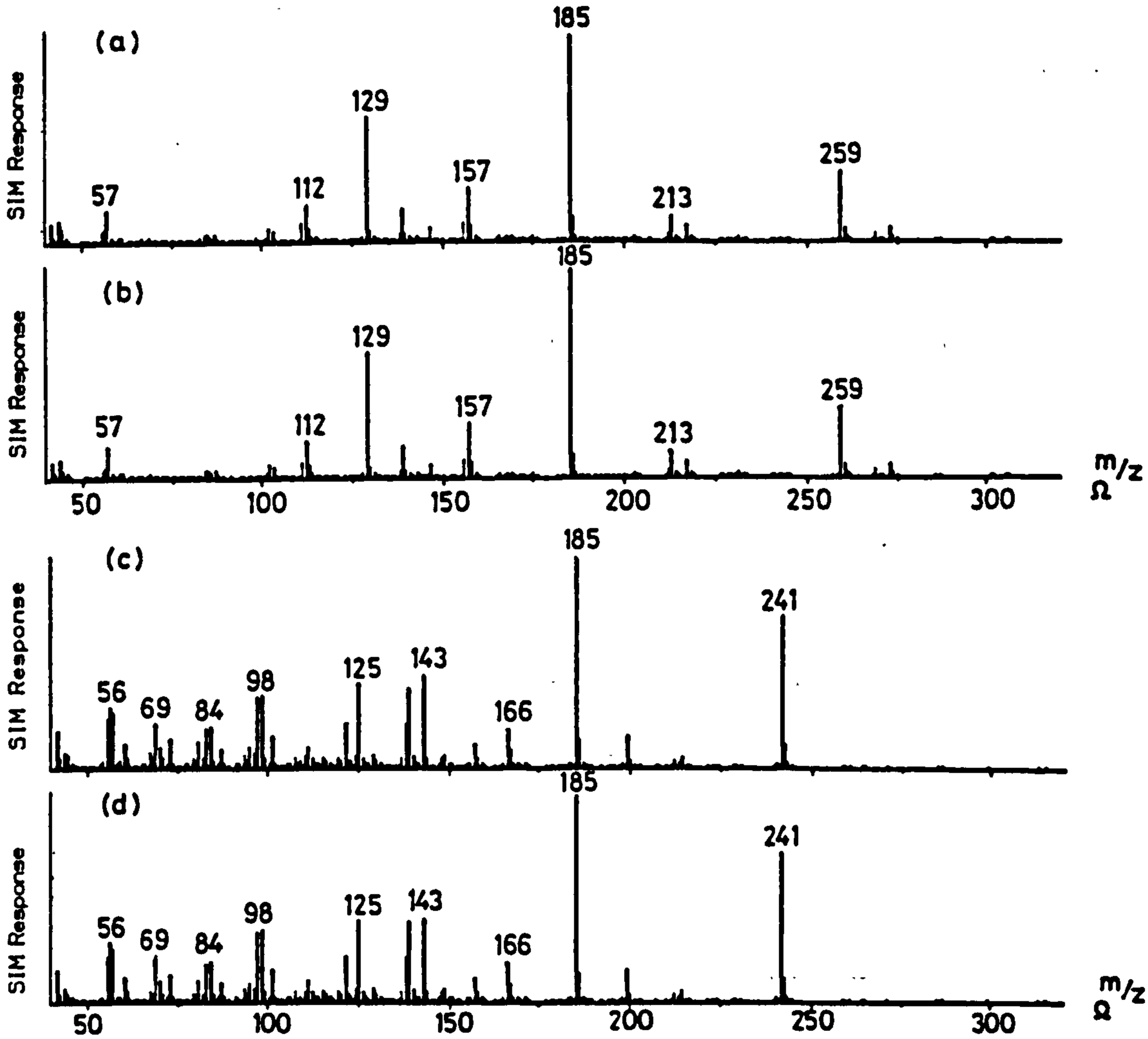
The identity of the ATBC and DBS peaks was confirmed by scanning GC-MS. For both plasticisers the agreement between the food sample, standard and a library spectrum was excellent (figure 8) indicating that no interferences co-eluted with either ATBC or DBS and thus establishing that quantification by GC-FID was reliable.

3.3.2 MIGRATION DATA

3.3.2.1 General Points

As was seen in section 2.3, reproducible migration data using real foods is difficult to obtain even under laboratory conditions. It was therefore anticipated that migration levels into seemingly identical retail food samples, where many of the parameters relevant to the migration process, including the storage history of samples prior to purchase, were unknown, would show a large degree of scatter. The analysis of six packets of minced beef wrapped in DEHA plasticised PVC which were apparently identical, having been purchased from the same outlet at the same time and being of identical pack size, had a RSD of 22%, table 6. This scatter can be attributed to variations in, the extent the film was stretched, the temperature experienced by each sample, whether it was stored at the bottom or top of the cool cabinet and the effect of stacking. Of the foods studied, the baked items and the broccoli were wrapped in film with no additional packaging material. All other samples were in over-wrapped trays.

Figure 8. Mass Spectral Confirmation of ATBC and DBS in Food.



(a) Spectrum identified as acetyl tributyl citrate (ATBC) obtained by GC-MS from a sample of processed cheese.
(b) Spectrum of ATBC standard obtained by GC-MS.
GC-MS conditions: 30m × 0·24mm I.D. DB5 fused silica column operated at a helium carrier gas flow rate of 1 mLmin^{-1} with temperature programming as follows: 215°C hold 6min, 20°C min^{-1} to 300°C hold 5 min.

(c) Spectrum identified as dibutyl sebacate (DBS) obtained by GC-MS from a sample of smoked cheese.
(d) Spectrum of DBS standard obtained by GC-MS.
GC-MS conditions: 30m × 0·24mm I.D. DB5 fused silica column operated at a helium carrier gas flow rate of 1 mLmin^{-1} with temperature programming as follows: 180°C hold 7 min, 20°C min^{-1} to 300°C hold 5 min.

The results for meat and poultry have been expressed in $mgkg^{-1}$ calculated for both the purchase weight (whole) and edible weight ("tissue") so that they can be used directly for dietary intake calculations. The results are also expressed in $mgdm^{-2}$ so that they are readily comparable to the results obtained in section 2.3. Once again, care should be taken when interpreting migration levels expressed in $mgdm^{-2}$, as a certain degree of estimation had to be employed when determining the contact area of foods of complex shapes such as chicken pieces or cornish pasties. The contact area was typically taken as the total surface area of the food which was approximated to one or more simple geometric shapes. The contact area of a cornish pastie, for example, was taken as the sum of two triangular sides and a rectangular base. This gave an over-estimate of the contact area as the cornish pastie had a central raised ridge of pastry which normally held the film away from certain areas of pastry. Also, for items packaged in deep sided trays, such as salads or stir-fried vegetables, contact with the film would only occur when the packs were stacked or tilted from the horizontal. In such cases the contact area was taken as the area of film available for contact.

3.3.2.2 Migration of DEHA

Previous work had indicated that there is no appreciable loss of DEHA from meat on cooking provided the meat juices were included in the analysis (107). Therefore, for the purpose of dietary intake calculations, it was decided that further preparation of uncooked food would be unnecessary and thus the migration level into the food as purchased was determined.

Migration into Meat

Migration levels into meat are given in table 6 and once again illustrate the importance of fat form and content. The highest level of migration observed, 64 $mgkg^{-1}$ (19 $mgdm^{-2}$), was into pork fat which is 72 % fat (121) and represented a 69% loss of DEHA from the film (19 $mgdm^{-2}$ from 28 $mgdm^{-2}$ available). High migration values (18-29 $mgkg^{-1}$, 8-11 $mgdm^{-2}$) were also observed into the pork loin joints (30 % fat (121)), expect for one sample (Loin joint ◊) which provides an interesting contrast. The migration level into this sample was only 2·0 $mgkg^{-1}$ (0·9 $mgdm^{-2}$). The contact surface of this sample was skin, which had been left on the joint, rather than fat in the case of the other pork loin samples.

The migration data obtained for the beef samples illustrate the influence of the distribution of fat on the degree of scatter for replicate samples. The RSD of the migration observed for braising steak was 12% compared to 77% for stewing steak. This difference would appear to be due to the difference in distribution of fat in the meat. In braising steak the fat is quite uniform whereas in stewing steak the contact area of fat for any given weight of meat is highly variable.

Table 6. Migration of DEHA into Meat from PVC.

Food Type	FILM DEHA (w%)	Wgt.(g) Whole	CA dm^2	DEHA MIGRATION $mgkg^{-1}$ Whole *	$mgdm^{-2}$	$mgkg^{-1}$ Tissue ¢
BEEF						
Braising steak	22	259	0·8	2·6	0·8	2·6
Braising steak	23	262	1·1	3·3	0·8	3·3
Braising steak	22	262	1·1	3·1	0·7	3·1
Minced beef	24	252	1·2	4·6	1·0	4·6
Minced beef	24	223	1·2	5·9	1·1	5·9
Minced beef	24	230	1·1	5·7	1·2	5·7
Minced beef	24	245	1·3	5·5	1·0	5·5
Minced beef	24	234	1·2	8·0	1·5	8·0
Minced beef	24	230	1·3	4·5	0·8	4·5
Stewing steak	22	380	1·4	2·1	0·6	2·1
Stewing steak	22	319	1·4	2·5	0·4	2·5
Stewing steak	22	301	1·3	7·8	1·8	7·8
Steak & kidney	22	319	0·9	3·1	1·1	3·1
Kidney	22	181	0·4	1·0	0·5	1·0
PORK						
Loin joint	18	896	2·0	18·4	8·2	23·0
Loin joint	20	770	2·1	29·0	10·7	36·3
Loin joint	20	865	2·0	22·8	9·7	28·5
Loin joint ◊	20	677	1·4	2·0	0·9	2·5
Hock	22	546	1·4	1·1	0·4	1·8
Hock	24	599	1·3	1·3	0·6	2·1
Fat	19	358	1·2	63·9	19·1	63·9
LAMB						
Leg steak	22	156	0·6	10·6	2·8	10·6
Breast	23	308	0·9	2·8	1·0	3·9
Stuffed breast	21	550	0·7	2·9	2·3	2·9

* "Whole" = Food as purchased
¢ "Tissue" = Food as eaten, i.e. boned
◊ See the text "Migration into Meat"

Migration into Chicken

Table 7 gives migration data for both uncooked and cooked chicken. The level of migration found in uncooked chicken was between 9 $mgkg^{-1}$ and 53 $mgkg^{-1}$ (2-9 $mgdm^{-2}$) calculated on the basis of the food as eaten, that is boned. The wide range of migration levels is probably due to the large variation in contact area pack size and amount of edible sample that was recovered after removal of bones rather than differences in the nature of the surface of the samples, which is relatively constant for uncooked chicken. An exception to this is a the sample of chicken breast Breast †. Two samples of chicken breast were analysed with approximately the same ratio of mass to contact area, however the migration levels were 19 $mgkg^{-1}$ (6 $mgdm^{-2}$) and 53 $mgkg^{-1}$ (18 $mgdm^{-2}$). This difference can be attributed to the fact that Breast † sample was not covered in skin and thus bare flesh was in contact with the film. The variation in migration levels for the cooked chicken can be attributed not only to the factors outlined above but also to the changes in the nature of the surface caused by cooking, or the presence of various sauces.

Table 7. Migration of DEHA into Chicken from PVC.

Food Type	FILM DEHA (w%)	Wgt.(g) Whole	CA dm^2	DEHA MIGRATION $mgkg^{-1}$ Whole *	$mgdm^{-2}$	$mgkg^{-1}$ Tissue ¢
CHICKEN						
Wings	19	315	0·8	6·8	2·5	11·3
Wings	23	475	1·3	6·3	2·3	10·5
Wings	24	294	0·8	7·4	2·6	12·7
Legs	23	383	1·0	8·6	3·4	11·0
Breast †	24	209	0·6	18·8	6·2	18·8
Breast	22	464	1·4	53·1	17·6	53·1
Drumsticks	21	340	0·2	5·7	8·1	8·5
Drumsticks	24	393	0·9	15·6	7·0	24·4
Thighs	24	266	0·8	27·3	9·4	33·2
Thighs	24	216	0·7	23·2	7·0	27·7
Leg quarter	25	223	0·5	9·6	4·0	13·9
Leg quarter	25	422	1·4	26·1	7·7	39·7
COOKED CHICKEN						
Barbecued	25	218	0·7	27·3	8·5	38·4
Barbecued	23	204	0·3	29·4	19·0	42·9
Tikka	24	227	0·8	33·5	9·5	48·6
Chinese style	25	400	1·0	8·1	3·2	12·1
Chinese style	25	482	0·3	8·7	15·0	13·7
Roast breast	24	181	0·7	27·4	7·1	27·4
Roast breast	23	245	0·5	7·1	3·2	10·3
Roast breast	25	174	0·4	29·3	12·1	42·4
Roast breast	24	223	0·3	6·5	4·8	9·4
Escalope	24	287	0·8	18·4	6·9	18·4
Breadcrumbed	23	255	1·2	72·8	15·5	72·8
Turkey burger	24	227	0·9	25·6	6·5	25·6
Tandoori	25	446	0·9	13·0	6·4	19·7
Tandoori	26	429	1·4	12·3	3·7	18·4
Roast leg	25	135	0·3	25·2	12·6	33·0

* "Whole" = Food as purchased
¢ "Tissue" = Food as eaten i.e. boned
† See the text "Migration into Chicken"

Migration into Cheese, Fruit and Vegetables, and Baked Goods

Data for cheese, fruit, vegetables and baked goods are given in table 8. At the time of this work relatively few examples of cheese wrapped in DEHA plasticised cling film were available. Those found invariably came from small retail outlets. The level of DEHA contamination found ranged from 28 to 135 $mgkg^{-1}$ which was consistent with published results (106).

Migration levels into fruit and vegetables were in agreement with those observed in section 2.3.2.3 and ranged between 0·3 and 1·8$mgkg^{-1}$ (0·04-0·3 $mgdm^{-2}$), excluding that into the stir-fry vegetables which was considerably higher and was dominated by the migration into the butter pat. Migration into apples was also slightly higher than might have been predicted, at 0·3-0·4 $mgdm^{-2}$, and may have been due to the practice of waxing the fruit to give it a shine.

The relatively high migration levels into the baked goods, 10 to 80 $mgkg^{-1}$ (2-7 $mgdm^{-2}$), can be attributed to the layer of surface fat often found on pastry and the high fat content of the cakes studied. The high level of migration, 30-130 $mgkg^{-1}$ (2-11 $mgdm^{-2}$), into the sandwiches and rolls were not anticipated on the basis of previous work (106) and is probably due to contact of the butter and filling with the film. Also, as the products were prepared commercially in large numbers, it seems quite likely that the surface of the bread may have received a thin coating of grease from buttered hands, or the stacking of buttered bread prior to the addition of the filling.

Table 8. Migration of DEHA into Cheese, Fruit, Vegetables and Baked Goods from PVC.

Food Type	FILM DEHA (w%)	Wgt.(g) Whole	CA dm^2	DEHA MIGRATION $mgkg^{-1}$	$mgdm^{-2}$
CHEESE					
Cherry coconut	18	103	1·1	135	12·8
Strong Cheddar	15	215	2·6	27·8	2·3
Red Cheddar	15	317	2·6	107	12·9
White Cheddar	14	293	2·6	88·4	10·0
Brie	22	184	2·2	114	9·5
FRUIT/VEG.					
Stir/fry veg. &	23	226	1·6	0·4	0·05
Garlic butter pat		16·6	0·1	57·5	7·6
Stir/fry veg. &	23	225	1·6	0·3	0·04
Butter pat		15·4	0·1	91·8	14·7
Salad items	23	200	2·0	0·4	0·04
Cox's Apples	22	703	1·1	0·6	0·4
G.Del. Apples	25	468	0·5	0·3	0·3
Broccoli (UK)	21	227	1·8	0·5	0·06
Broccoli (Esp.)	24	227	1·8	0·6	0·07
Broccoli (Cape)	20	227	1·5	1·8	0·3
BAKED GOODS					
Cornish pastie	24	453	3·3	11·3	1·6
Sausage rolls	22	260	2·7	59·9	5·8
Cheese & onion roll	12	128	3·1	72·8	3·0
Sardine roll	13	94	2·9	121·0	3·9
Corned beef roll	21	144	2·7	30·0	1·6
Fruit scone	13	76	1·5	54·0	2·7
Jam doughnut	12	72	1·3	74·9	4·3
Cheese sandwich	20	100	1·2	129·8	10·7
Swiss roll	15	338	4·4	69·4	5·4
Battenburg	17	359	3·5	33·1	3·4
Cherry cake	13	428	4·7	79·0	7·2

G.Del. Apples Golden Delicious Apples

3.3.2.3 Migration of DBP, DCHP, BBP and DPOP

Three types of nitrocellulose-coated RCF were found; those plasticised with DBP alone, those with DBP and DCHP, and those with DBP, DCHP, BBP and DPOP. The level of each plasticiser in the films was typically 0·5 -1·5% on a total film-weight basis. Levels of the individual plasticisers are given in tables 9 and 10, and these represent approximately 30-40% plasticiser in the nitrocellulose coating. The shape and nature of the goods packaged in the films, meat pies and pasties for example, meant that accurate determination of contact area was not practical. Therefore, when calculating the migration levels in terms of $mgdm^{-2}$, the total area of film available for contact was taken.

Meat pies and pasties show migration values in the range 2-10 $mgkg^{-1}$ (0·2-1·5 $mgdm^{-2}$). The specific weights of these RCF films were 320-390 $mgdm^{-2}$ and each individual plasticiser was therefore available at around 2-6 $mgdm^{-2}$. As both sides of RCF is coated with nitrocellulose, each side has 1-3 $mgdm^{-2}$ available plasticiser and therefore migration values at the upper end of the range seen represent considerable depletion from the film.

Table 9. Migration of DBP and DCHP into Foodstuffs from Nitrocellulose-coated RCF.

Food Type	Wgt. (g)	CA (dm^2)	Film (w.%) DBP	Film (w.%) DCHP	Phthalate Migration $mgkg^{-1}$ DBP	Phthalate Migration $mgkg^{-1}$ DCHP	Phthalate Migration $mgdm^{-2}$ DBP	Phthalate Migration $mgdm^{-2}$ DCHP
Maltesers	42	1·6	0·5	--	12·4	--	0·3	--
Nougat	175	3·2	0·1	--	0·5	--	0·03	--
Fudge	30	0·6	0·3	--	3·6	--	0·2	--
Cornish Pastie	152	0·5	0·6	0·7	1·2	1·1	0·4	0·3
Crusty Pork Pie	120	1·4	0·6	0·8	5·5	6·2	0·5	0·5
Cornish Pastie	149	2·0	0·7	0·8	8·7	6·7	0·6	0·5
Steak & Kid. Pie*	154	0·9	1·6	1·8	7·8	8·6	1·3	1·4
Steak & Kid. Pie*	173	0·9	1·1	1·8	6·2	5·4	1·1	1·0
Steak & Kid. Pie*	163	0·9	1·1	1·7	7·5	7·4	1·3	1·3
Cornish Pastie*	167	1·8	1·4	1·6	15·6	16·9	1·4	1·6
Flake	36	1·2	1·2	1·5	30·8	19·8	0·9	0·6
Mixed boiled Sweets (21 different)	183	ND	0·3	0·5	7·0	6·7	--	--
Sultana Cake	391	3·7	0·7	1·0	15·2	14·8	1·6	1·6

CA Food-film contact area
ND Not Determined
* These pies had been stored in their packaging in a hot display cabinet

A few confectionery products were examined and, as might have been anticipated, the virtually fat-free nougat gave, essentially, a blank result. This was in contrast to the chocolate-coated products which were contaminated at levels of 4-31 $mgkg^{-1}$ (0·2-0·6 $mgdm^{-2}$). Once again the level of migration into sandwiches was relatively high at 10-16 $mgkg^{-1}$ (0·4-0·7 $mgdm^{-2}$). The explanation for this is the same as for the high level of DEHA migration from PVC cling film, that is the contact of filling and butter with the film.

Table 10. Level of DBP, DCHP, BBP and DPOP in Nitrocellulose-coated RCF.

Food Type	Wgt. (g)	CA (dm^2)	Film Composition (wgt. %) DBP	DCHP	BBP	DPOP
MEAT PIES						
Potato,Cheese & Onion Pastie	135	0·8	0·7	0·8	0·6	0·5
Chicken & Veg. Pie	133	0·9	0·7	0·7	0·5	0·5
Sausage Rolls	114	0·5	1·0	1·0	0·8	0·6
Country Pork Pie	121	1·4	0·6	0·8	0·5	0·5
Scotch Eggs	243	0·4	0·5	0·5	0·4	0·3
Premium Steak & Mushroom Pie	186	0·9	0·6	0·6	0·5	0·3
Chicken & Mushroom Pie*	139	0·9	0·8	0·8	0·7	0·6
SANDWICHES						
Cheese & Tomato	124	2·7	0·6	0·7	0·6	0·3
Egg Mayonnaise	103	2·3	0·6	0·5	0·5	0·3
Cheese & Pickle	119	2·5	0·5	0·6	0·5	0·3

Table 11. Migration of DBP, DCHP, BBP and DPOP into Foodstuffs from Nitrocellulose-coated RCF.

Food Type	$mgkg^{-1}$ DBP	DCHP	BBP	DPOP	$mgdm^{-2}$ DBP	DCHP	BBP	DPOP
MEAT PIES								
Potato,Cheese & Onion Pastie	4·6	1·6	1·5	0·8	0·8	0·3	0·3	0·1
Chicken & Veg. Pie	3·2	1·6	1·5	0·7	0·4	0·2	0·2	0·1
Sausage Rolls	5·0	2·0	2·1	0·8	1·2	0·5	0·5	0·2
Country Pork Pie	9·5	12·0	12·0	6·2	0·8	1·0	1·0	0·5
Scotch Eggs	2·2	0·9	1·0	0·4	1·3	0·5	0·6	0·2
Premium Steak & Mushroom Pie	3·1	0·2	1·2	0·2	0·6	--	0·2	--
Chicken & Mushroom Pie*	8·0	9·1	4·5	3·4	1·3	1·5	0·7	0·5
SANDWICHES								
Cheese & Tomato	12	16	14	8·5	0·6	0·7	0·7	0·4
Egg Mayonnaise	13	16	15	8·6	0·6	0·7	0·7	0·4
Cheese & Pickle	11	14	13	9·4	0·5	0·7	0·6	0·4

CA Food-film contact area
* This pie had been stored in its packaging in a hot display cabinet

3.3.2.4 Migration of ATBC

The retail use of ATBC plasticised films is generally confined to to the over-wrapping of "blocks" of cheese. In all cases the level of ATBC in the film was around 4·5% by mass. The levels of ATBC found in cheese are given in table 12 and range from 1·3 to 7·7 $mgkg^{-1}$ (0·1-0·6 $mgdm^{-2}$). The mass per unit area (grammage) of the films was around 250-270 $mgdm^{-2}$, thus the available ATBC was 11-12 $mgdm^{-2}$ and therefore the migration levels observed represent 1-6% loss of ATBC from the film.

Table 12. Migration of ATBC into Cheese from PVDC.

Food Type	Wgt. (g)	CA (dm^2)	Film ATBC(%)	ATBC Migration $mgkg^{-1}$	$mgdm^{-2}$
White Cheshire	216	3·6	4·5	2·9	0·2
Brie, full fat	145	1·6	4·4	1·5	0·1
Brie, full fat	137	1·7	4·7	3·3	0·3
Danish Blue	150	1·5	4·5	2·4	0·2
Danish Blue	113	1·6	4·3	7·7	0·6
Emmental with Walnuts	131	1·0	4·3	6·0	0·7
Cheese Assortment					
a) Brie	123	1·6	4·5	1·6	0·1
b) Mature Cheddar	111	1·6	4·5	5·9	0·4
c) Smoked Processed	77	0·4	None	--	--
d) Blue Brie	100	1·3	4·3	2·3	0·2
Total	411			2·6	0·2
Bavarian Processed	164	1·8	4·3	3·2	0·3
Camembert, full fat	142	1·9	4·5	1·3	0·1
French Roule	131	1·2	4·5	5·0	0·5
Chevre Blanc (Goats)	99	1·0	4·4	3·6	0·4
Chevre Blanc (Goats)	106	1·2	4·4	4·4	0·4

CA Food-film contact area

3.3.2.5 Migration of DBS

Only four products were found in DBS plasticised films, two types of cheese and two meat products. The level of DBS in the film and grammage were similar to that for ATBC plasticised film, at approximately 4 % and 260 $mgdm^{-2}$ respectively. The film was used either to over-wrap blocks of cheese, as in the St. Paulin cheese, or in "chub packs" as in the smoked cheese and meat products. The migration into the St. Paulin cheese was low at 2-4 $mgkg^{-1}$ (0·2-0·4 $mgdm^{-2}$) and comparable to that of ATBC into cheese.

The migration levels into the "chub packed" products was much higher at 76 to 137 $mgkg^{-1}$ (8 to 15 $mgdm^{-2}$) and represented the loss of approximately one third of the available DBS. This high level of migration is attributable to the reported practice of heat sterilising the meat product in the packaging material (122) combined with the effects of a relatively thick film, a fatty product and a long shelf life.

Table 13. Migration of DBS into Cheese and Cooked Meat Products from PVDC.

Food Type	Wgt. (g)	CA (dm^2)	Film DBS(%)	DBS Migration $mgkg^{-1}$	$mgdm^{-2}$
CHEESE					
French St. Paulin	163	1·8	4·4	3·6	0·3
French St. Paulin	198	1·9	4·1	3·9	0·4
French St. Paulin	194	1·9	4·1	3·3	0·3
French St. Paulin	202	1·9	4·0	2·3	0·2
Smoked Processed	71	0·7	3·9	85	8·5
Smoked Processed	71	0·7	3·5	101	10·1
Smoked Processed	71	0·7	3·7	76	7·6
Smoked Processed	71	0·7	3·8	112	11·2
MEAT PRODUCTS					
Liver Pate	113	1·0	3·9	89	10·2
Liver Pate	113	1·0	3·9	97	11·1
Liver Pate	113	1·0	4·0	132	15·1
Liver Pate	113	1·0	3·5	89	10·1
Black Pudding	170	1·5	4·2	137	15·1
Black Pudding	170	1·5	3·7	109	12·0
Black Pudding	170	1·5	4·0	116	12·8
Black Pudding	170	1·5	4·2	125	13·8

CA Food-film contact area

3.3.2.6 Summary of Results

The level of plasticiser contamination of food resulting from the retail use of plasticised packaging material is influenced by the same factors effecting the migration of DEHA into food under simulated domestic use. That is, the fat form and content of the food, the temperature and length of exposure of the food to the film and the manner in which the food is wrapped. That is, whether it is completely over-wrapped in film, over-wrapped on a polystyrene tray or a deep-sided tray where the food is not intended to come into direct contact with the film.

CHAPTER 4

MATHEMATICAL MODELLING OF THE MIGRATION OF DEHA FROM PVC CLING FILM INTO FOOD

4.1 INTRODUCTION

It is evident that a predictive mathematical model of additive migration could reduce the number of experiments required to provide data for dietary intake calculations and could assist in setting priorities for migration experiments by identifying those additives most likely to migrate. The large amount of experimentation required to assess just a handful of additives (plasticisers) migrating from a limited number of polymer systems (chapters 2 and 3) could not be repeated for the many hundreds of additives on the impending European Community positive list and the dozens of polymer systems therein.

A number of mathematical models for additive migration from polymers into food are available in the literature (123). Few of these models have, however, been validated by a comparison of predicted and experimentally determined levels of migration into real foods under realistic exposure conditions. It was, therefore, appropriate to assess an existing model rather than develop a new model.

Till *et al* (124) proposed equation 1 to predict additive migration from plastic packaging into food. It appeared a suitable model for the prediction of plasticiser migration from PVC cling film into food. The migration of DEHA into food was studied since the required migration data was available (106, 107).

$$M_t = 2C_{P_o}\left(\frac{D_p\ t}{\pi}\right)^{1/2}\ \frac{\beta}{1+\beta} \qquad (1)$$

M_t - mass of additive migrated from the polymer in time t
C_{P_o} - original concentration of the additive in the polymer
t - time

β allows for any resistance to additive migration that might occur in the food and is given by :-

$$\beta = K\left(\frac{D_f}{D_p}\right)^{1/2}$$

K - partition coefficient of additive between polymer and food
D_f - diffusion coefficient of the additive in the food
D_p - diffusion coefficient of the additive in the polymer

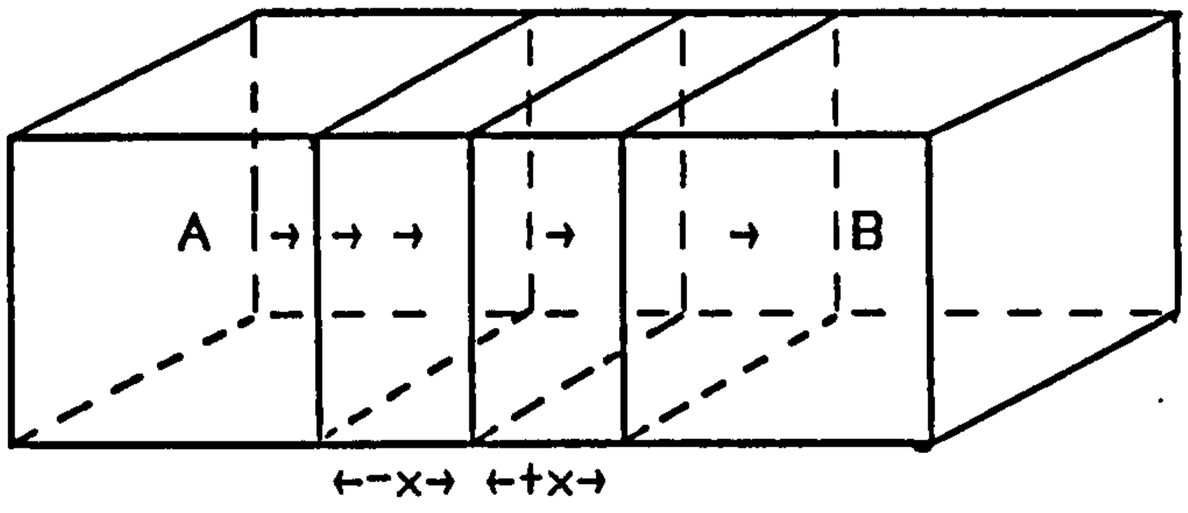
Of the five variables in Till's model, C_{P_o} and t are readily obtained experimentally and D_p for DEHA in PVC is available in the literature (125). No values of D_f in a real food or K between a polymer and a real food are available, Till *et al.* used estimated values in their work (125). Thus values for D_f for DEHA in a food and K of DEHA between a food and PVC cling film were required for the thorough evaluation of Till's model. Before discussing suitable methods of determining D_f and K it is appropriate to discuss the phenomenon of diffusion.

4.1.1 INTRODUCTION TO THE THEORY OF DIFFUSION

Diffusion is the transport of molecules through a system due to random molecular motion. The phenomenon may be explained by considering a system containing two homogeneous gases A and B which are separated by a barrier. When the barrier is removed the gases are free to mix. Consider the movement of A through a plane perpendicular to the x axis

and two small equal elements of volume just to the left (-x) and right (+x) of that plane (figure 9).

Figure 9. The Movement of Gas A Through a System Containing Two Gases, A and B, Initially Separated by a Barrier.



Because the motion of the molecules is random it is not possible to predict which molecules will pass through the plane during any given time period. On average, however, a given fraction of the molecules in each element of volume will pass from -x to +x or from +x to -x respectively. Since the concentration of A is greater in region -x than +x there will be a net transfer of A from -x to +x. That is from the region of high concentration to the region of low concentration. Thus even though the motion of the molecules is random, because there is a concentration gradient, A is transported throughout the system.

The amount of A, or any diffusing substance, that is transferred through a plane in a given time period, that is the flux (F), is dependent on the rate of change of concentration of A with distance (x). This may be represented mathematically by equation 2. Equation 2 only applies if the composition of the diffusing substance is homogeneous with respect to the y and z axis.

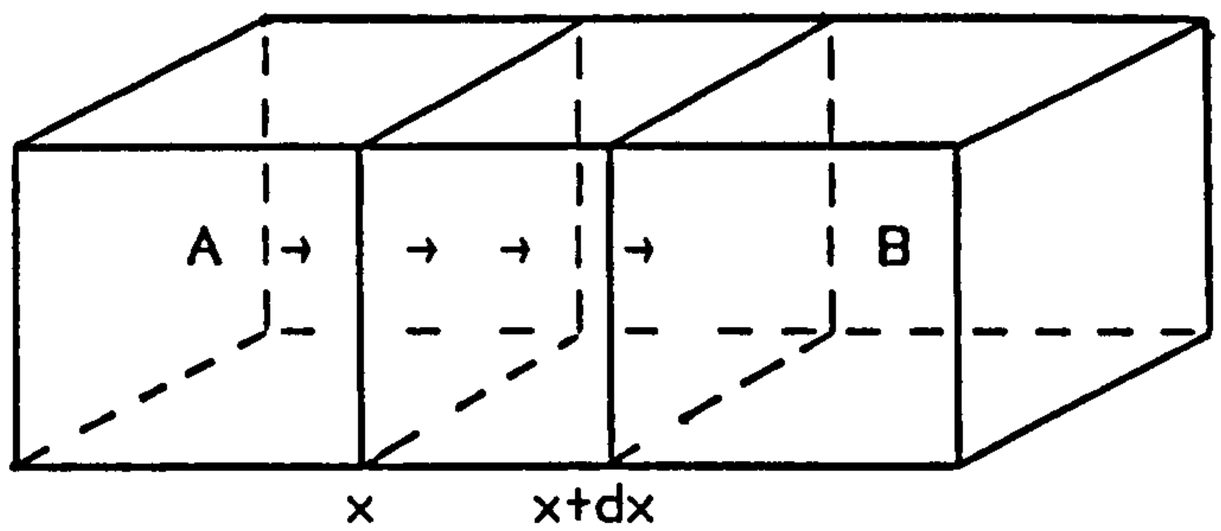
$$F \propto \frac{dC}{dt} \qquad (2)$$

Adding a constant of proportionality, the diffusion coefficient (D), to equation 2 gives Fick's First Law of Diffusion, equation 3. The negative sign is a result of diffusion occurring in the opposite direction to increasing concentration of the diffusant. Fick's first law applies only to diffusion in a steady state, that is where concentration at a given point does not vary with time.

$$F = - D \frac{dC}{dx} \qquad \text{Fick's First Law of Diffusion} \quad (3)$$

For a non-steady state system the flux of A through a volume element of unit area between x and x + dx, figure 10, equals the mass of A transported into the volume element minus that which is transported out, divided by the volume. This can be expressed mathematically by equation 4.

Figure 10. Non-Steady State Diffusion of Gas A through a System Containing Two Gases, A and B, Initially Separated by a Barrier.



$$\frac{dC_A}{dt} = \frac{1}{dx}\,[F_A(x) - F_A(x+dx)] \qquad (4)$$

Since $$F_A(x+dx) = F_A(x) + \frac{dF_A}{dx}\,dx$$

$$\frac{dC_A}{dt} = -\frac{dF_A}{dx}$$ and from equation 3

$$\frac{dC_A}{dt} = \frac{d}{dx}\,D\,\frac{dC_A}{dx} \qquad (5)$$

If D is independent of x equation 5 may be expressed as

$$\frac{dC_A}{dt} = D\left(\frac{d^2C_A}{dx^2}\right) \qquad (6)$$

The above equation is a truncated expression of Fick's second law of diffusion as it only considers diffusion in one dimension (x). The full expression of Fick's second law is obtained by carrying out a similar treatment for the transport of A in all three dimensions (x,y,z) (equation 7).

$$\frac{dC_A}{dt} = D\left(\frac{d^2C_A}{dx^2} + \frac{d^2C_A}{dy^2} + \frac{d^2C_A}{dz^2}\right) \qquad \text{Fick's Second Law of Diffusion} \quad (7)$$

The mathematical solution of Fick's second law is dependant on the boundary conditions of the system under consideration. By selection of the appropriate mathematical solution the concentration of a diffusant at any given point after any given period of time may be calculated.

Solutions of Fick's second law for an extensive range of boundary conditions have been reported by Crank (127).

4.1.2 DETERMINATION OF THE DIFFUSION COEFFICIENT OF DEHA IN A REAL FOOD

Due to the complex nature of the solutions of Fick's second law, diffusion coefficients are typically determined by a process of graph fitting rather than by numerical analysis. Plots of the concentration of the diffusant (C) with distance (x) at a given time (t) or C versus t at a given value of x are typically used (128,129,130). C versus x at a fixed value of t was plotted in this work.

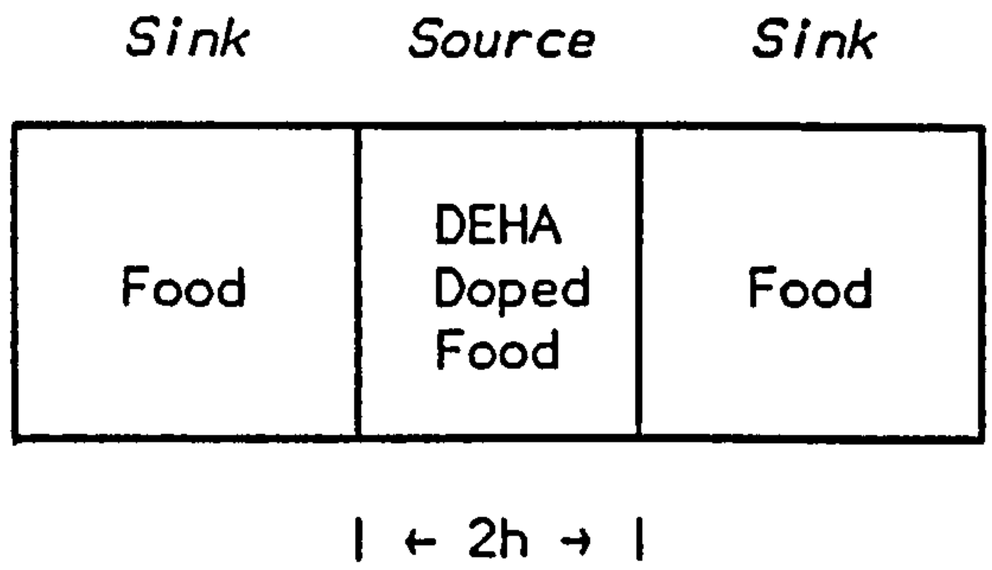
The sample geometry given in figure 11 was selected for the determination of the diffusion coefficient of DEHA in food so that the relatively simple solution of Fick's second law of diffusion, given in equation 8 (127), could be used in the graph fitting process.

$$C = \frac{C_o}{2}\left\{\operatorname{erf}\left(\frac{h-x}{2(Dt)^{1/2}}\right) + \operatorname{erf}\left(\frac{h+x}{2(Dt)^{1/2}}\right)\right\} \qquad (8)$$

C = concentration of migrant
C_o = initial concentration of migrant
D = diffusion coefficient of migrant
t = time
h = half the thickness of the source
x = distance from the origin O where O = mid-point of the source

erf = error function where $\operatorname{erf} z = \frac{2}{\sqrt{\pi}}\int_0^z \exp(-\eta^2)d\eta$

Figure 11. Cross-Section of Sample Used to determine the Diffusion Coefficient of DEHA in a Real Food.



The use of the same food as the source and the sink eliminates the possibility of the nature of either being significantly changed by counter diffusion of compounds from the sink into the source or vice versa. This would complicate the interpretation of the DEHA concentration profiles obtained.

It was decided to use Cheddar cheese as the test food as it is relatively homogeneous and its surface and bulk properties are similar. Thus the junction between the DEHA doped and undoped cheese was a single discrete interface rather than a series of boundary layers. The high solubility of DEHA in Cheddar cheese, as illustrated by the high migration levels reported in the literature (103,106,107) and chapters 2 and 3, ensured that the undoped cheese could be considered, mathematically, as an infinite sink which is a requirement of equation 8. In addition, Cheddar cheese is a solid which facilitates sample preparation and handling.

4.1.3 DETERMINATION OF THE PARTITION COEFFICIENT OF DEHA BETWEEN PVC CLING FILM AND CHEESE

The determination of the partition coefficient of DEHA between Cheddar cheese and PVC cling film ($K_{DEHA\ cheese/film}$) is complex due to the intractable nature of the materials. The classical method of determining partition coefficients by measuring the equilibrium concentration of analyte in two matrices is not practical for the measurement of $K_{DEHA\ cheese/film}$. The time required for such a system to equilibrate is excessive. From equation 9 (131) it would take approximately 1,600 years for DEHA to diffuse 1 cm^2 ($D = 10^{-11}\ cm^2s^{-1}$ (19)).

$$\bar{x}^2 = 2Dt \qquad (9)$$

$\bar{x}^2$ - mean square distance diffused
D - diffusion coefficient
t - time

Thus $K_{DEHA\ cheese/film}$ can only be determined indirectly. Cheese may be considered to consist of three components, water, lipid and solid. The partition coefficient of DEHA between each component and a common liquid phase, such as acetonitrile (ACN), can be measured and a value of $K_{DEHA\ cheese/ACN}$ derived on the basis of the summation of the component values. Once $K_{DEHA\ film/ACN}$ has been measured a value for $K_{DEHA\ cheese/film}$ can be calculated.

After establishing the diffusion coefficient of DEHA in Cheddar cheese and the partition coefficient of DEHA between Cheddar cheese and PVC cling film it was possible to evaluate Till's model (equation 1).

4.2 EXPERIMENTAL

4.2.1 MATERIALS

4.2.1.1 Primary and Internal Standards and Solvents

DCHP (British Cellophane Ltd.) and DEHA (Hexaplas DOA, ICI) were commercially available and better than 96% pure when analysed by capillary GC. All solvents were of HPLC-grade and supplied by Rathburn (Walkerburn, Scotland) unless stated otherwise.

4.2.1.2 Cheddar Cheese

Cheddar cheese doped with DEHA was prepared at the Food Science Department, Reading University. DEHA (80 mL) was added to pasteurized milk (182 L, 40 gallons) and mixed thoroughly to ensure a homogeneous starting material. Tests had previously been conducted to ensure that the addition of DEHA at this level would not kill the starter mix which was a culture of lactic acid organisms. The cheese was then made according to a standard procedure for the production of Cheddar cheese (132).

Red Cheddar cheese was purchased from a local retail outlet where it had previously been established that the product had not been in contact with DEHA plasticised packaging. The absence of DEHA in the cheese was confirmed by GC analysis.

4.2.2 INSTRUMENTATION

Homogenizer, Ultra-Turrax; Cooled Incubator, Baird & Tatlock; Cryostated microtome, SLEE London; Grinder, Coffee Grinder, Braun; Oven, Ove-205-010H Gallenkamp.

4.2.3 DETERMINATION OF THE DIFFUSION COEFFICIENT OF DEHA IN CHEDDAR CHEESE

4.2.3.1 Level of DEHA in Doped Cheese

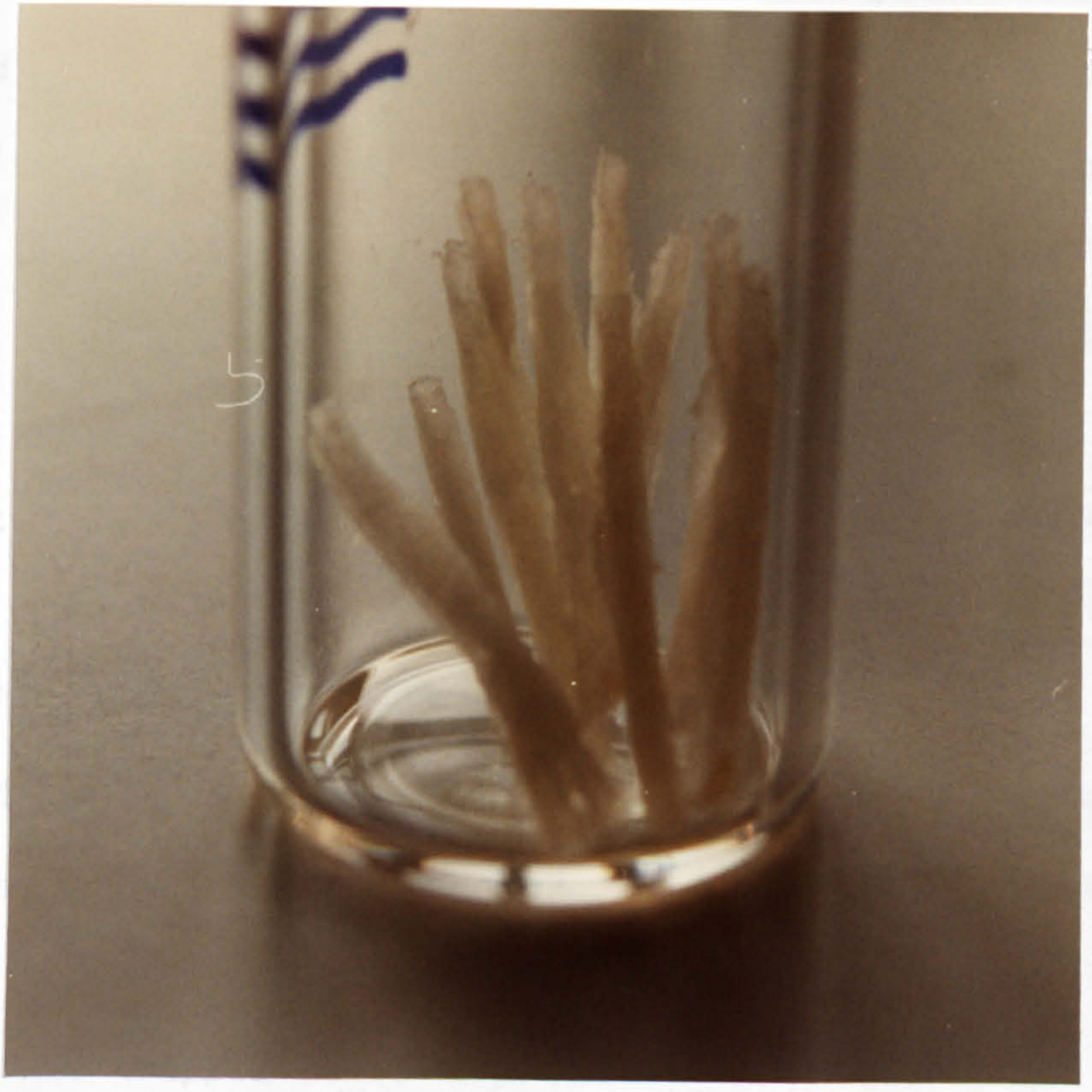
Triplicate sub-samples (10g) of the doped cheese were weighed accurately and homogenized in acetone/hexane (1:1 v/v, 100 mL) after the addition of DCHP (20 mg) in acetone/hexane (1:1) as an internal standard. The mixture was set aside for two hours to allow the internal standard and analyte to equilibrate. The organic layer was decanted from the residue which was then re-extracted with a further portion of solvent (100 mL). The combined extract was dried (Na_2SO_4) and then analysed using the GC system described in section 3.2.2 and the following temperature program; 210°C for 1 min; 4°Cmin^{-1} to 220°C and hold 1 min; 20°Cmin^{-1} to 300°C and hold 1 min. Quantification was based on peak area of DEHA relative to the internal standard.

4.2.3.2 Homogeneity of DEHA in the Doped Cheese

A cylinder of doped cheese (ca. 18mm × 11mm) was prepared by boring a glass tube, inner diameter 11 mm, through a block of doped cheese and then expelling the cheese with a Teflon™ plunger. The cheese was then

mounted on a microtome chuck in embedding medium (OCT Compound, Raymond A. Lamb) and placed in the cabinet of the cryostated microtome, set at -40°C, to cool. Once the cheese had become rigid the sample was installed in the microtome and sequential slices (20µm) taken. The anti-roll bar was removed so that each slice rolled into a small cylinder and could then be placed into a vial (figure 12). The temperature in the microtome cabinet was found to be critical. Above -30°C the cheese did not slice cleanly and the slices did not roll into a cylinder. As the temperature increase above -30°C the cheese began to crumble and ultimately deformed away from the blade and it was not possible to slice.

Figure 12. 20µm Slices of Cheddar Cheese in 1·5 mL Vial.



A series of individual slices, groups of ten sequential slices or ten sequential slices interspersed with 40 or 50 discarded slices, were collected into vials (1·5 mL) such that approximately 13 mm in total of the cheese was sectioned. The vials were chilled at -40°C to aid sample handling by preventing the slices fouling the neck of the vial. DEHA was then extracted from the samples by shaking overnight in acetone/hexane (1:1 v/v, 1 mL), containing DCHP as an internal standard. The extract was then analysed directly from the vial by GC using the system outlined in section 3.2.2 and an isothermal column temperature of 225°C.

4.2.3.3 DEHA Concentration Profile in Cheese Diffusion Experiment.

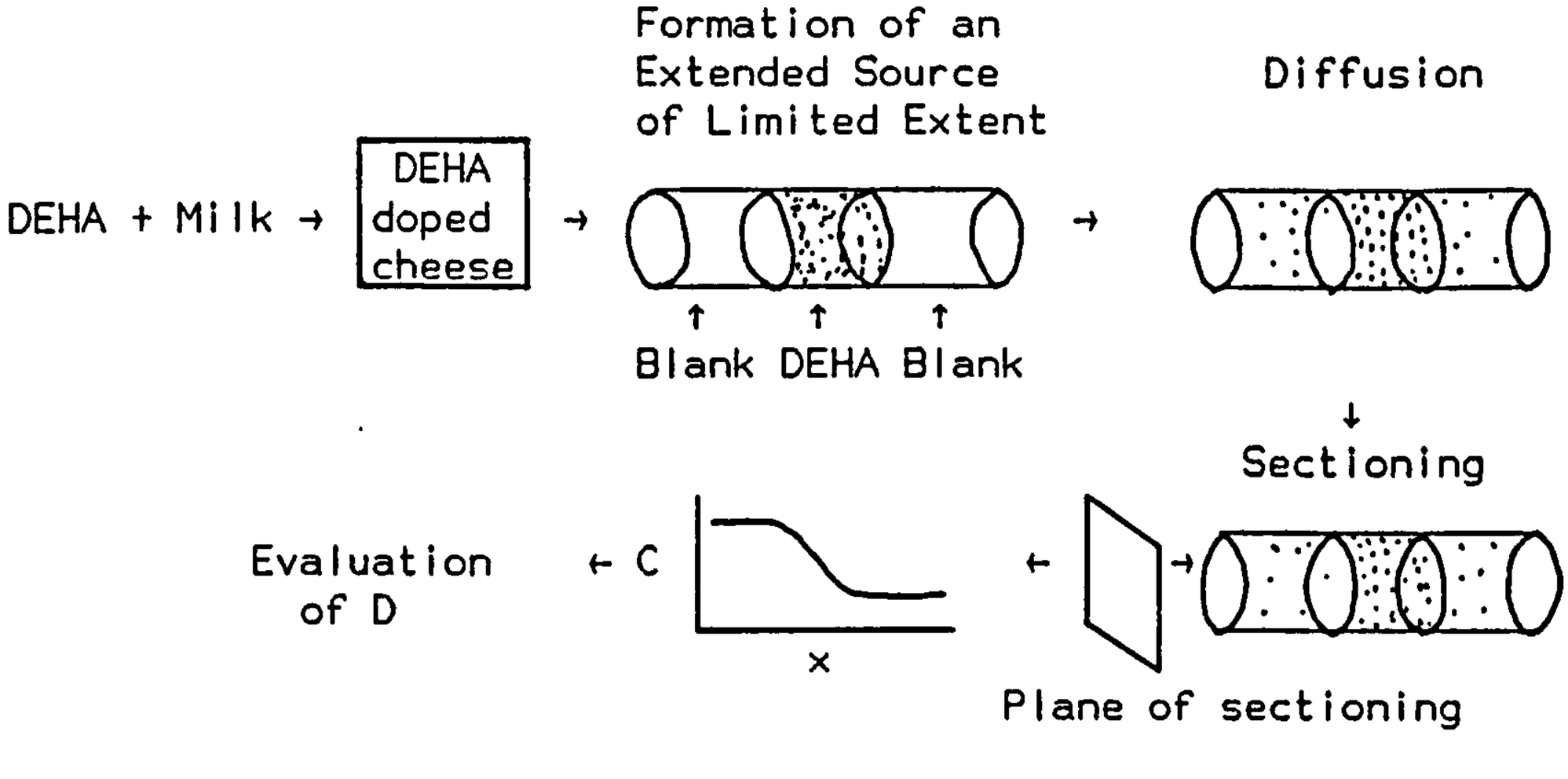
The determination of the concentration profile of DEHA which had diffused from doped cheese into undoped cheese is represented schematically in figure 13. Doped Cheddar cheese was sandwiched between two slices of undoped red Cheddar cheese. The interface between the doped (yellow) and undoped (red) cheese was clearly visible. The DEHA was then allowed to diffuse for a set time at a set temperature and the resultant concentration profile determined.

Sample Preparation and Exposure

A cylinder of undoped/doped/undoped cheese (figure 14) was prepared by boring a glass tube (11 mm I.D., 14 mm O.D.) sequentially through undoped (15mm), doped (8mm) and undoped cheese (15mm). To ensure that air was not trapped between the sections of cheese the sampling tube was pushed through the cheese such that approximately 1mm of cheese protruded. This cheese then made good contact with the next block of

cheese to be sampled. Duplicate samples were prepared and stored at either 5°C or 25°C ± 0·2°C for six and four days respectively in an incubator. The precise exposure time was noted.

Figure 13. Schematic Diagram of the Determination of the Concentration Profile of DEHA Diffusing Cheese.



Sampling

After exposure the glass tube was warmed gently and the cheese expelled with a Teflon` plunger. A portion containing a section of doped cheese, the doped/undoped cheese interface and a section of the undoped cheese (figure 15), was then mounted on a chuck in embedding medium and placed in the cabinet of a cryostated microtome, set at -40°C, to cool. Once the cheese had become rigid the sample was installed in the microtome, sliced (20 μm) and either 2, 5, or 10 sequential slices placed in vials (1·5 mL). In a region remote from the doped/undoped interface 10, 40 or 50 slices were discarded between the collection of sequential slices. The collection and sampling rate was such that the majority of slices collected were from the region of the interface. Once again the vials were chilled and the anti-roll bar was not used during sectioning to facilitate sample handling.

Figure 14. Cheese Cylinder Prepared for the Determination of the DEHA Concentration Profile in Undoped Cheese Exposed to Doped Cheese.

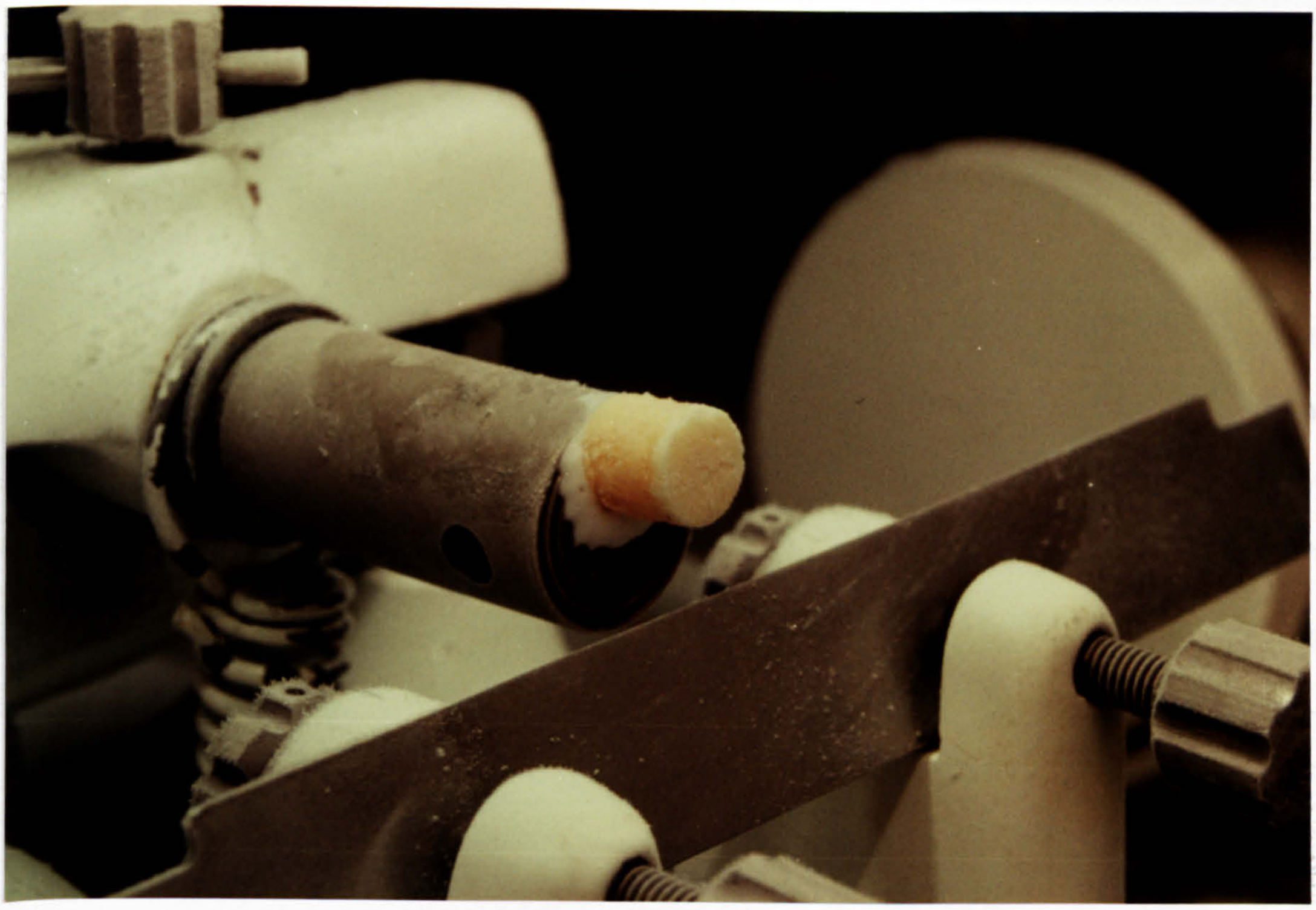
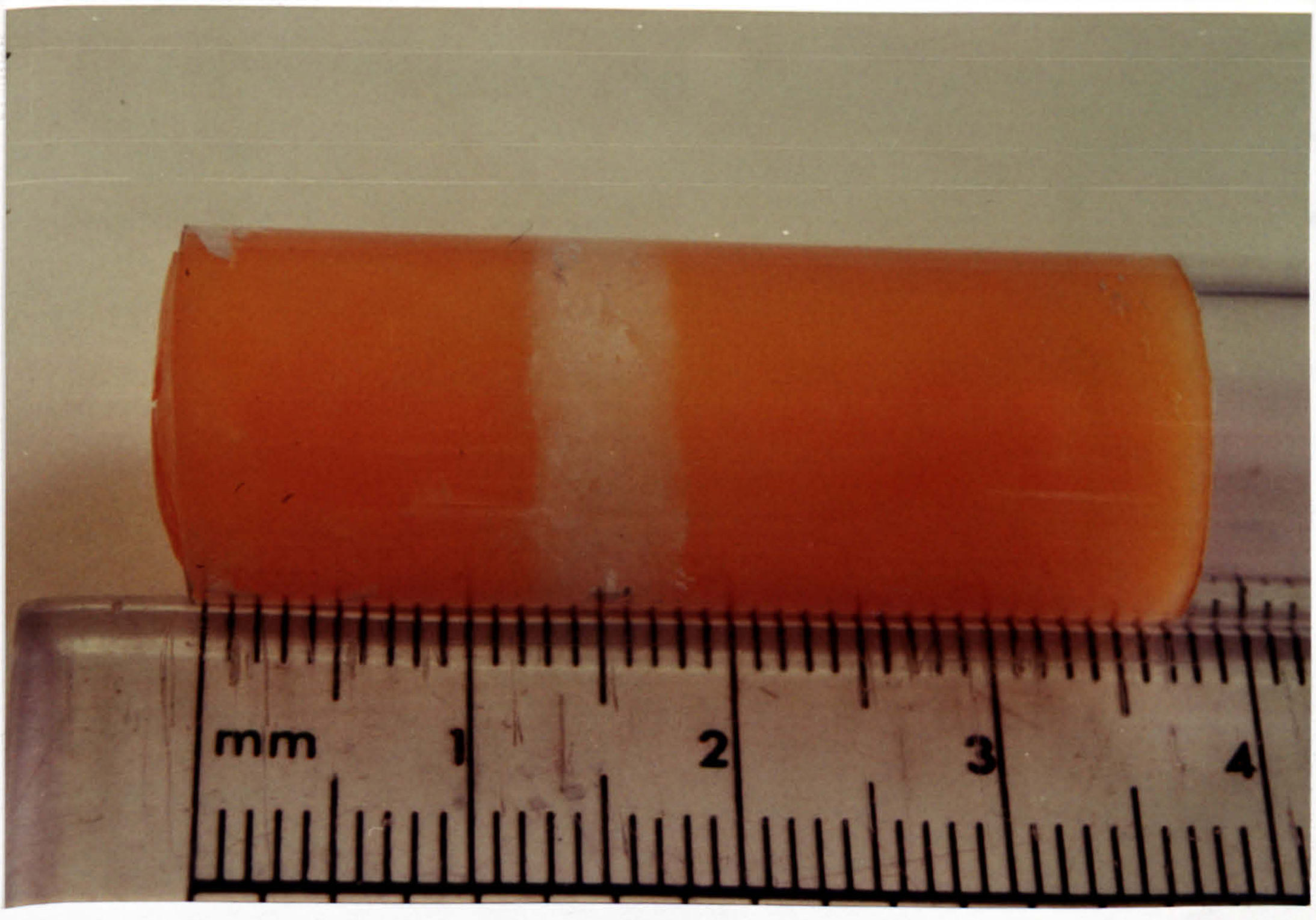


Figure 15. Portion of Cheese Cylinder Taken for Sectioning and Mounted in the Microtome.



Extraction and Analysis

Acetone/hexane (1:1 v/v, 1 mL), containing DCHP (20 µg) as an internal standard, was added to the samples which were shaken overnight. The extract was then analysed directly from the vial by GC as described in section 4.2.3.2

4.2.3.4 Determination of the Diffusion Coefficient of DEHA in Cheese

After establishing the DEHA concentration profile in the doped and undoped cheese the diffusion coefficient was obtained by graph fitting calculated concentration profiles to the experimental data. The concentration profiles were calculated using a Mallard BASIC computer programme, given in the appendix, for the evaluation of equation 8, and run on an Amstrad PCW8512 personal computer. The value of C_o, h, x and t for each sample was known and D was varied until the best visual fit was obtained.

4.2.4 DETERMINATION OF THE PARTITION COEFFICIENT OF DEHA BETWEEN CHEDDAR CHEESE AND PVC CLING FILM

As discussed earlier (section 4.1.3) the partition coefficient of DEHA between Cheddar cheese and PVC cling film can only be determined indirectly. The partition coefficients of DEHA between cheese lipid and acetonitrile (ACN), cheese solid and ACN, and PVC cling film and ACN were determined. The partition coefficient of DEHA between water and ACN was not determined as DEHA has a low solubility in water. The partition coefficient of DEHA between Cheddar cheese and PVC cling film was then calculated.

4.2.4.1 Partition Coefficient of DEHA between Cheddar Cheese Lipid and Acetonitrile

Triplicate samples of cheese lipid (3 g), which had been extracted from Cheddar cheese with acetone/hexane (1:1 v/v) and dried (Na_2SO_4), were placed in a vial (20 mL) and DEHA (1·5 mg) in ACN (3g) added. The samples were then allowed to come to equilibrium in an incubator at 5°C or 25°C. The attainment of equilibrium was established by analysis of the ACN phase for DEHA with time. Periodically an aliquot (100 µL) of the organic phase was removed, added to a solution of DCHP (0·05 mg), as an internal standard, in ACN (1 mL) and analysed for its DEHA level by GC using the method given in section 4.2.3.2. The system was kept at constant volume by the addition of fresh solution of DEHA in ACN (100µL, 0·5 $mgmL^{-1}$).

Once the system had come to equilibrium an aliquot (100 µL) of the lipid phase was removed, added to a solution of DCHP (6 µg) in acetone/hexane (1:1 v/v, 1 mL) and analysed for DEHA.

4.2.4.2 Partition Coefficient of DEHA between Cheddar Cheese Solid and Acetonitrile

The lipid was extracted from a portion of Cheddar cheese with acetone/hexane (1:1 v/v) and the resulting solid dried to constant weight at 65°C. The cheese solid was ground to a fine powder to ensure it was homogeneous. Triplicate sub-samples (3g) of the cheese solid were placed in vials (20 mL) containing a solution of DEHA (1·5 mg) in ACN (3 g) and allowed to come to equilibrium in an incubator at 5°C or 25°C. The system was kept at constant volume and the equilibration process was monitored, as described above.

4.2.4.3 Partition Coefficient of DEHA Between PVC Cling film and Acetonitrile

Triplicate samples of PVC cling film (18% DEHA plasticised w/w, 50 mg) were placed in vials containing ACN (3 g) and held in an incubator at 5°C or 25°C. The system was kept at constant volume and the equilibration process was monitored as described in section 4.2.4.1.

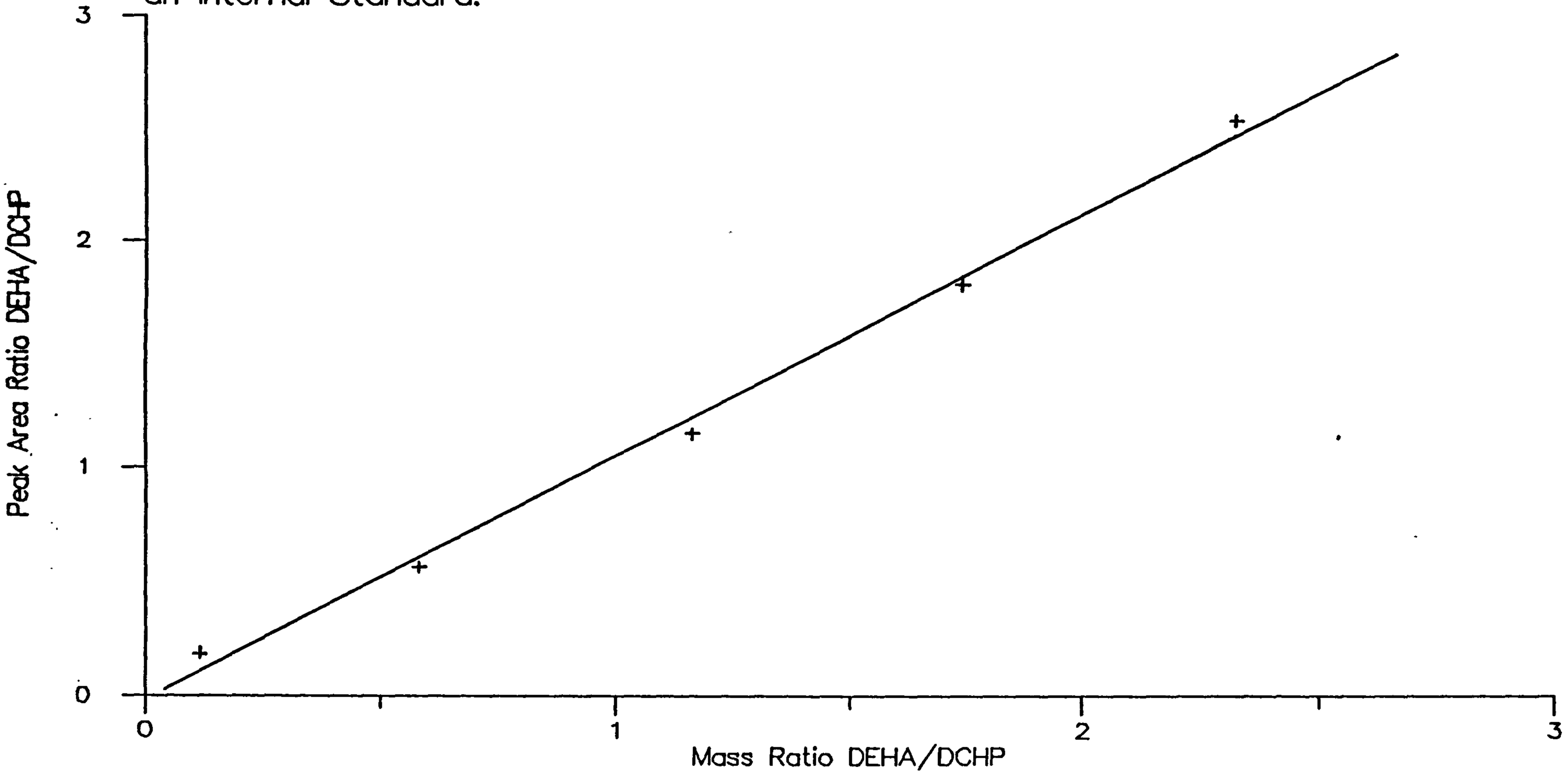
Once the system had reached equilibrium the film was removed, dried with a tissue and extracted with chloroform (4 mL) by shaking overnight. DCHP (0·1 mg) in ACN (0·5 mL) was then added to the chloroform extract which was analysed for DEHA as in section 4.2.3.2.

4.3 RESULTS AND DISCUSSION

4.3.1 ANALYTICAL METHODOLOGY

The determination of the level of DEHA in milligram cheese samples was based on the method of analysis for DEHA in food (104) used in chapters 2 and 3. It utilised DCHP as an internal standard and involved solvent extraction with acetone/hexane (1:1, v/v) followed by direct analysis of the extract by GC-FID. The sample size and high concentration of DEHA incorporated into the doped cheese, ca. 2,000 $mgkg^{-1}$, meant that the use of SEC clean-up and GC-MS end determination was unnecessary. A straight line calibration graph (correlation coefficient of 0·9974 and slope of 1·07) for mixtures of DEHA/DCHP for concentration ratios in the range 0:1 to 2·5:1 (figure 16) was obtained illustrating the suitability of DCHP as an internal standard. The RSD of the method was

Figure 16. Calibration Graph for the Analysis of DEHA by GC using DCHP as an Internal Standard.



Slope = 1.07
Correlation Coefficient = 0.9974
Intercept (x = 0) = −0.02

2% based on the analysis of replicate (n = 3) samples of cheese spiked at 2,000 $mgkg^{-1}$ taken through the entire method.

4.3.2 DETERMINATION OF THE DIFFUSION COEFFICIENT OF DEHA IN CHEDDAR CHEESE

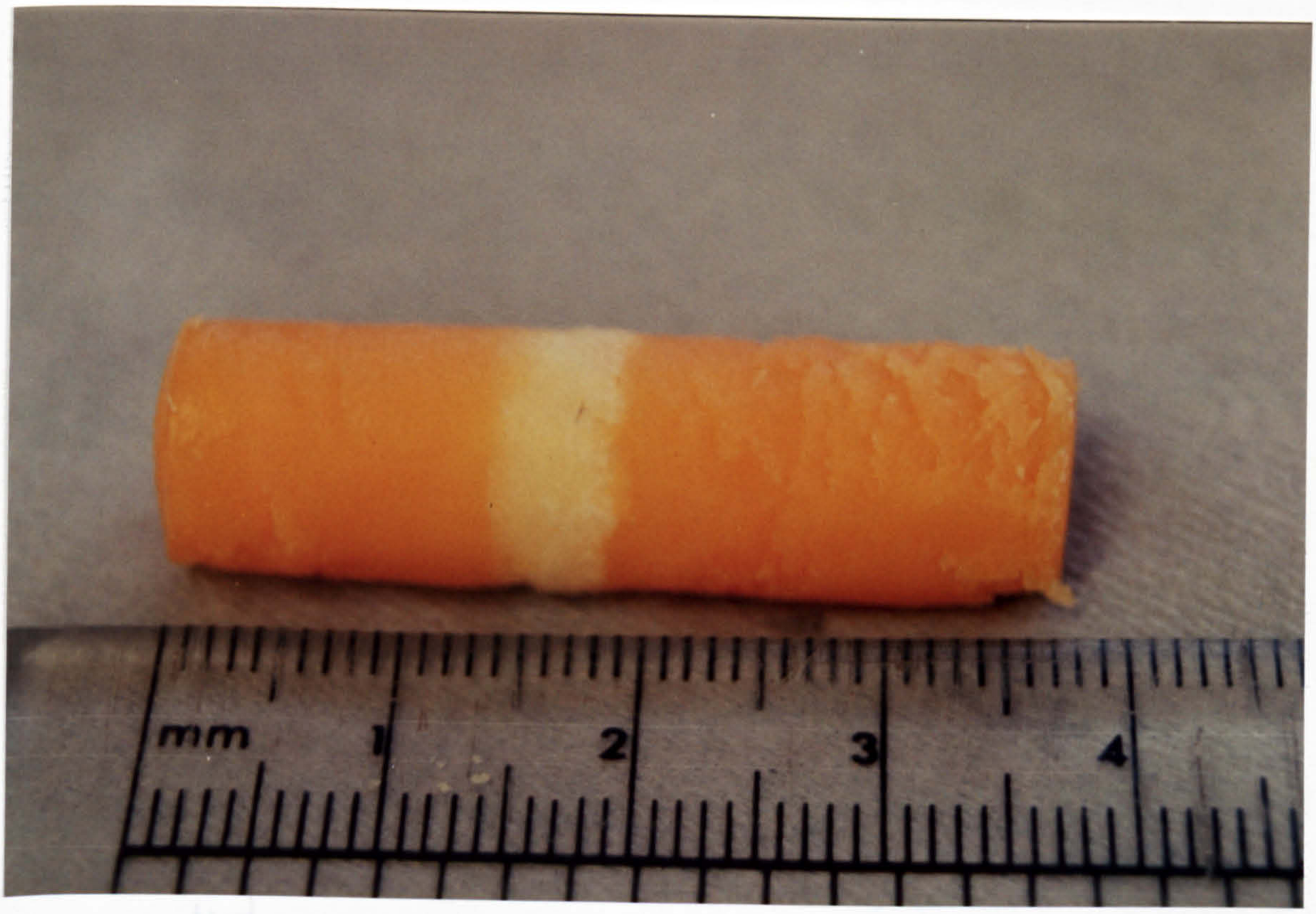
4.3.2.1 Concentration of DEHA in Doped Cheese

The mean concentration (n = 3) of DEHA in the doped cheese was 1,930 $mgkg^{-1}$. This was sufficiently low for the doped cheese to be considered a dilute solution of DEHA and thus D is independent of DEHA concentration. The DEHA concentration was nevertheless sufficiently high that the percentage loss of DEHA due to diffusion into the undoped cheese was small and thus the doped cheese could be considered, mathematically, an infinite source which is a requirement of equation 8.

4.3.2.2 Homogeneity of DEHA in the Doped Cheese

DEHA is relatively inhomogeneously distributed in the doped cheese on a micrometer scale with a RSD of 26% (n = 20) for sequential 20µm slices. The high RSD is due to inhomogeneity in the cheese structure as illustrated by figures 23, 25, and 27 and discussed in section 4.3.2.3. The distribution of DEHA on the millimetre scale is considerably more uniform: the RSD for the analysis of ten sequential slice taken at 0·2 mm, 1mm and 1·2 mm intervals was 9% (n = 10), 6% (n = 5) and 13% (n = 4) respectively.

Figure 17. Cheese Sample Removed from Tube Prior to Sectioning.



4.3.2.3 Concentration Profile of DEHA Diffused From Doped Cheese into Undoped Cheese

It can be seen from figure 17 that the contact between the undoped and doped cheese was intimate and that the sharpness of the interface between the doped and undoped cheese was preserved throughout the experiment.

The mean diffusion coefficients of DEHA in Cheddar cheese, as determined by graph fitting, were $1{\cdot}5 \times 10^{-9}$ cm^2s^{-1} at 5°C (figure 18 & 19) and 3×10^{-8} cm^2s^{-1} at 25°C (figure 20 & 21). These values are reasonable given that D for DEHA in corn oil is 10^{-7} cm^2s^{-1} at 4°C (125) and, from the determination of the partition coefficient of DEHA

Figure 18. Comparison of Measured and Calculated Concentration Profiles of DEHA which has Diffused from DEHA Doped Cheese into Undoped Cheese Exposed for Six Days at 5C.

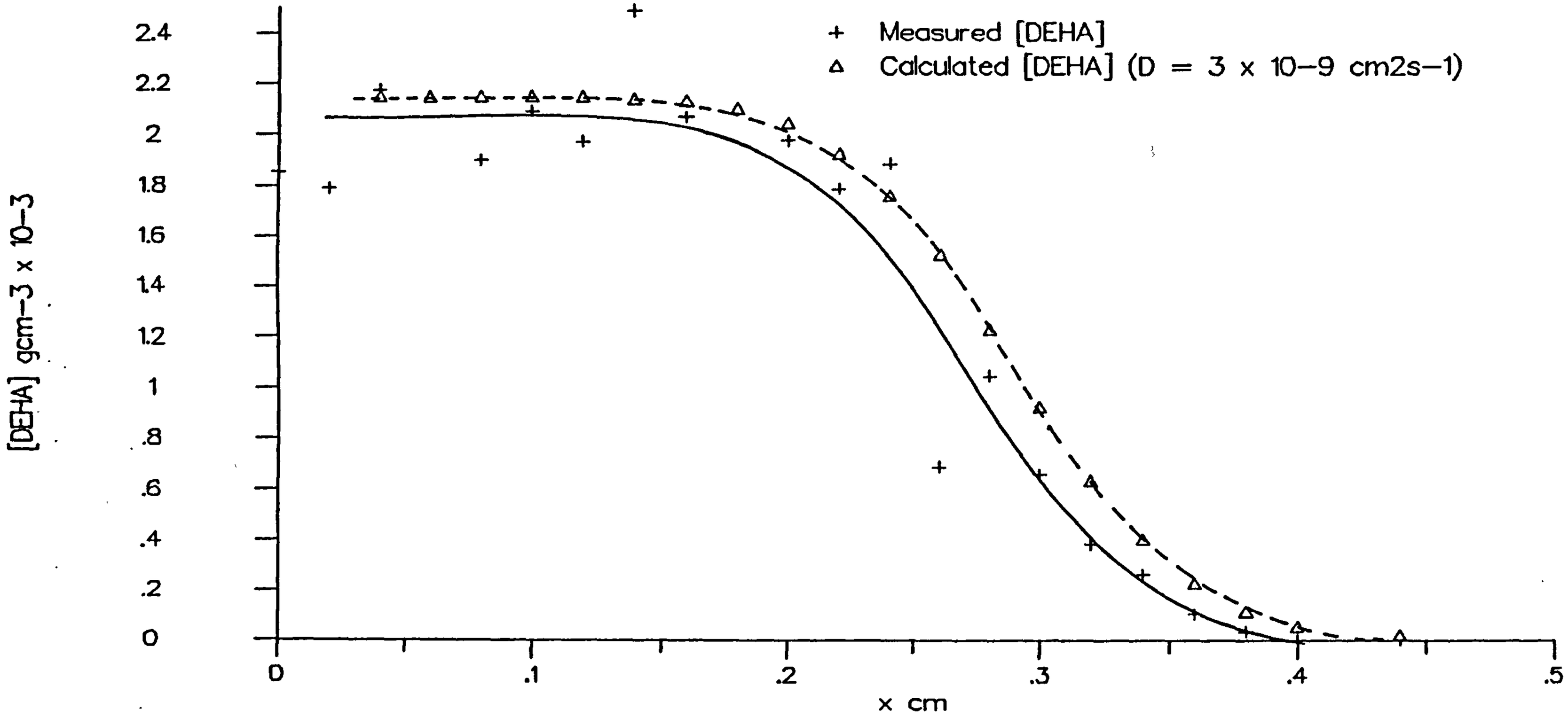


Figure 19. Comparison of Measured and Calculated Concentration Profiles of DEHA which has Diffused from DEHA Doped Cheese into Undoped Cheese Exposed for Six Days at 5C.

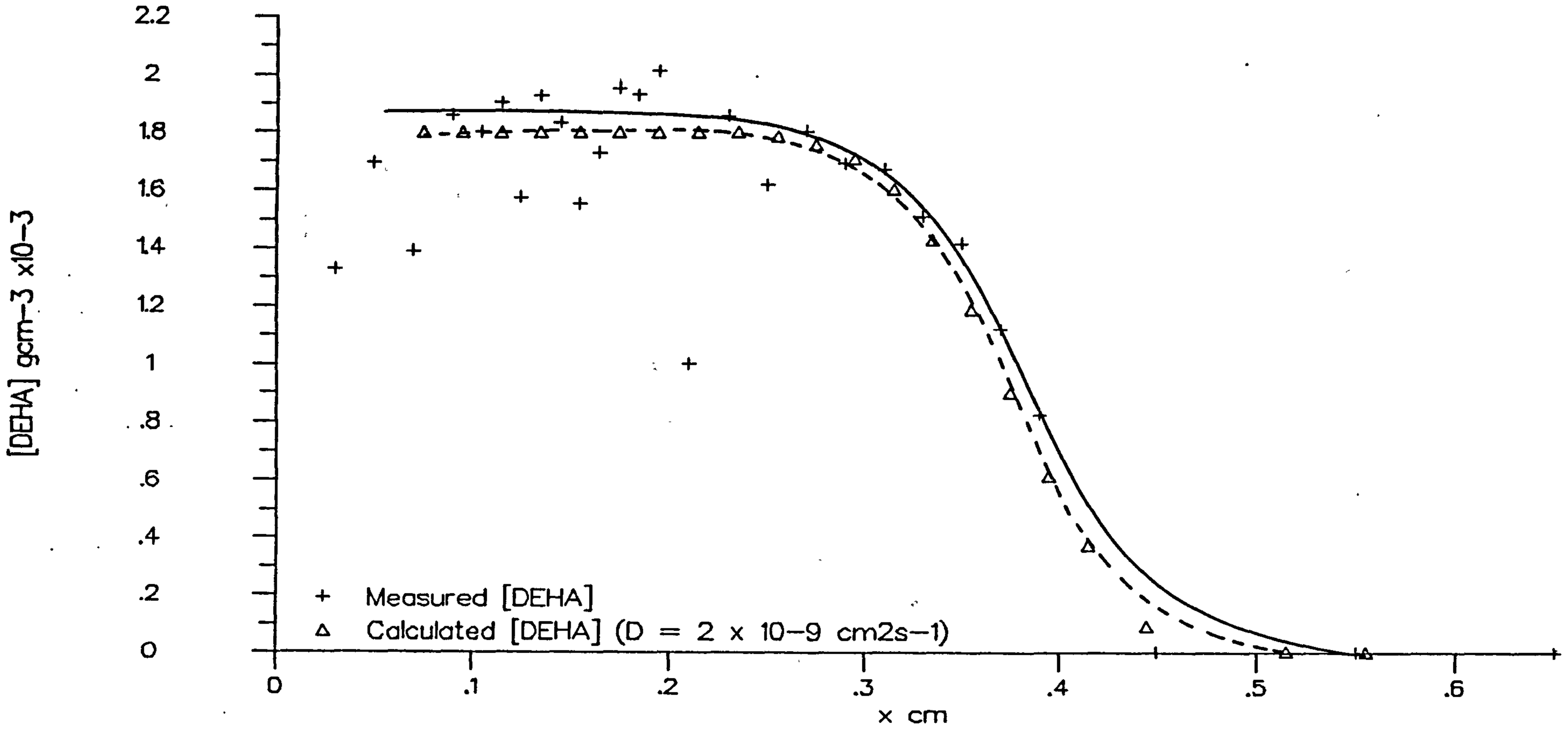


Figure 20. Comparison of Measured and Calculated Concentraton Profiles of DEHA which has Diffused from DEHA Doped Cheese into Undoped Cheese Exopsed for Four Days at 25C.

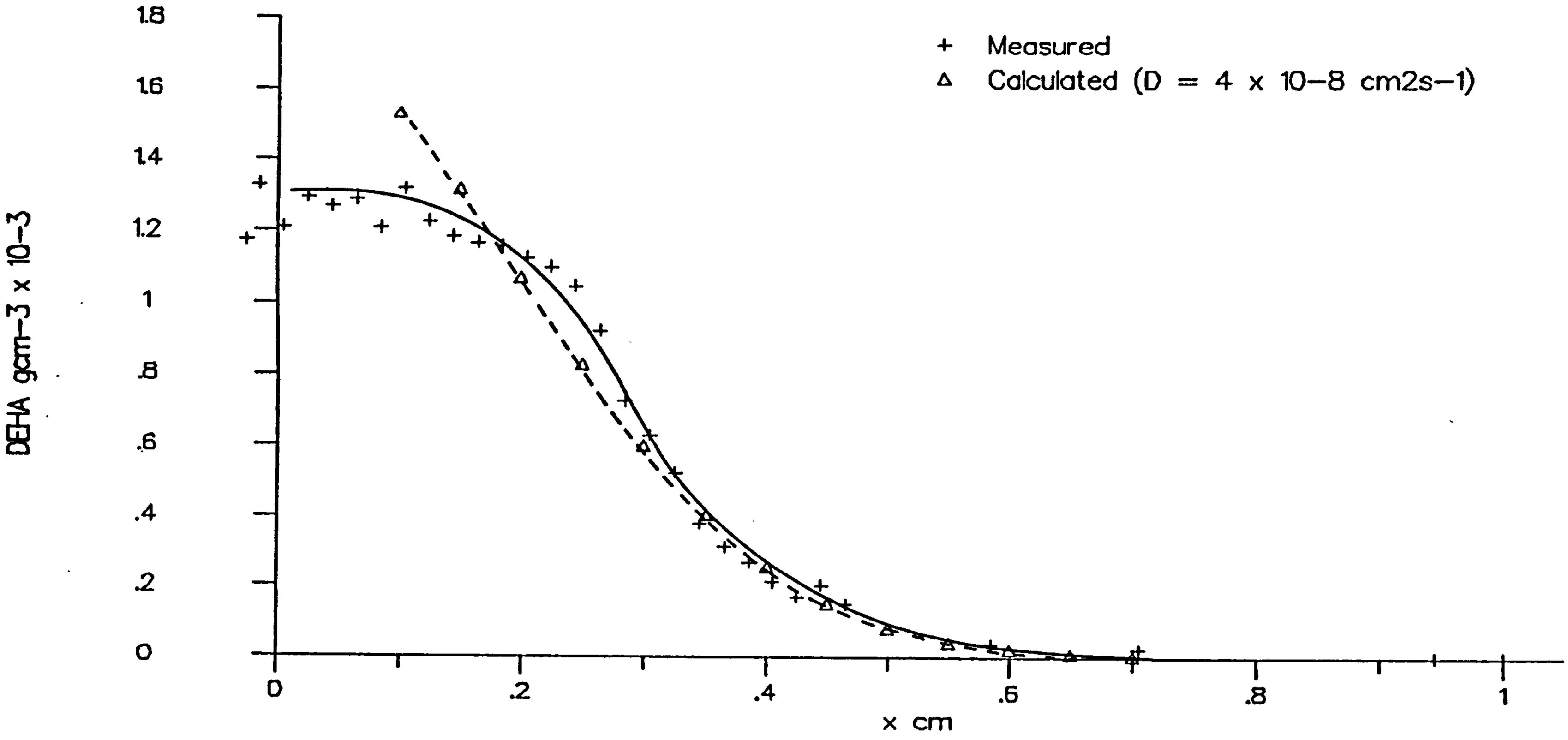
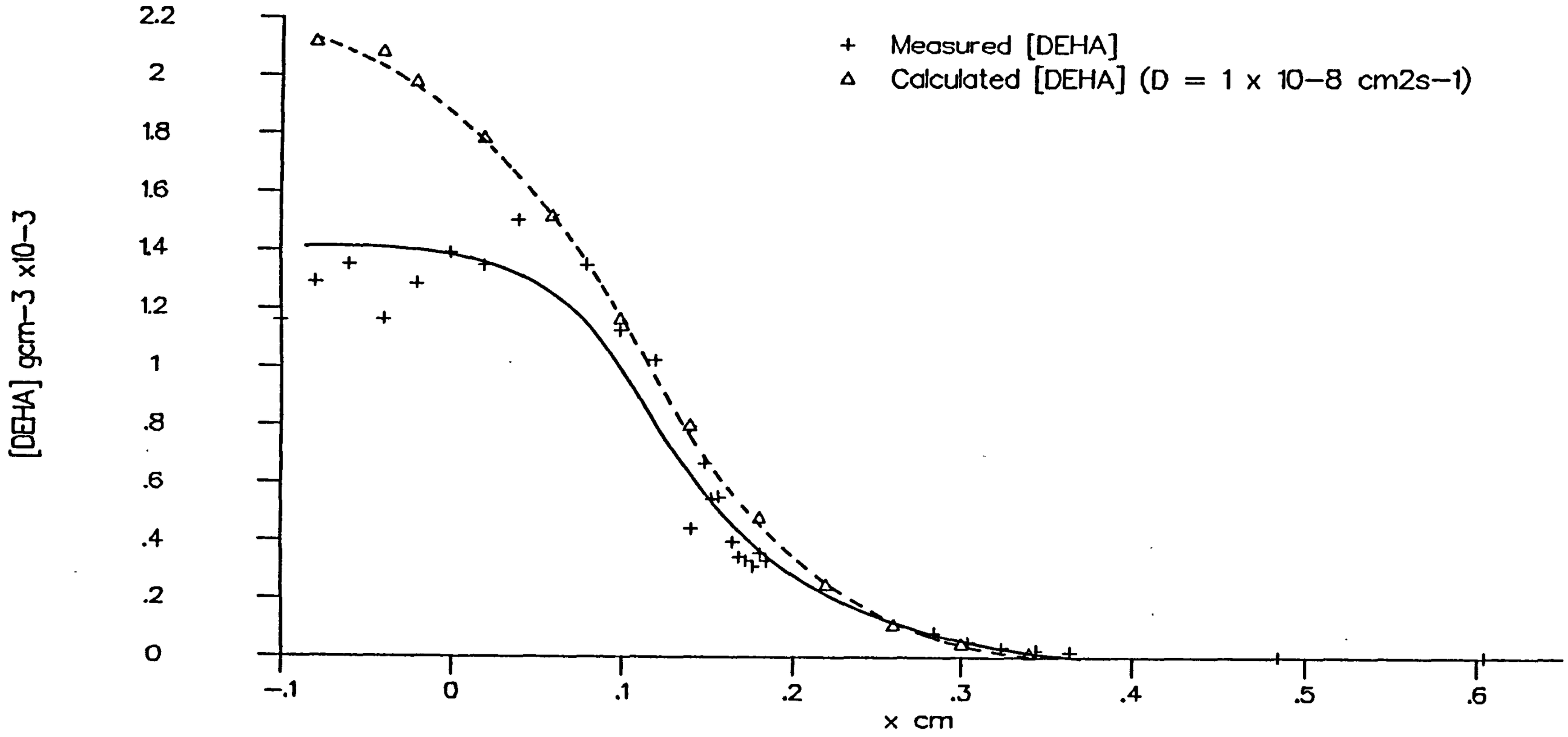


Figure 21. Comparison of Measured and Calculated Concentration Profiles of DEHA which has Diffused from DEHA Doped Cheese into Undoped Cheese Exposed for Four Days at 25C.



between cheese and cling film (section 4.3.2.4) DEHA diffuses through cheese via the lipid phase.

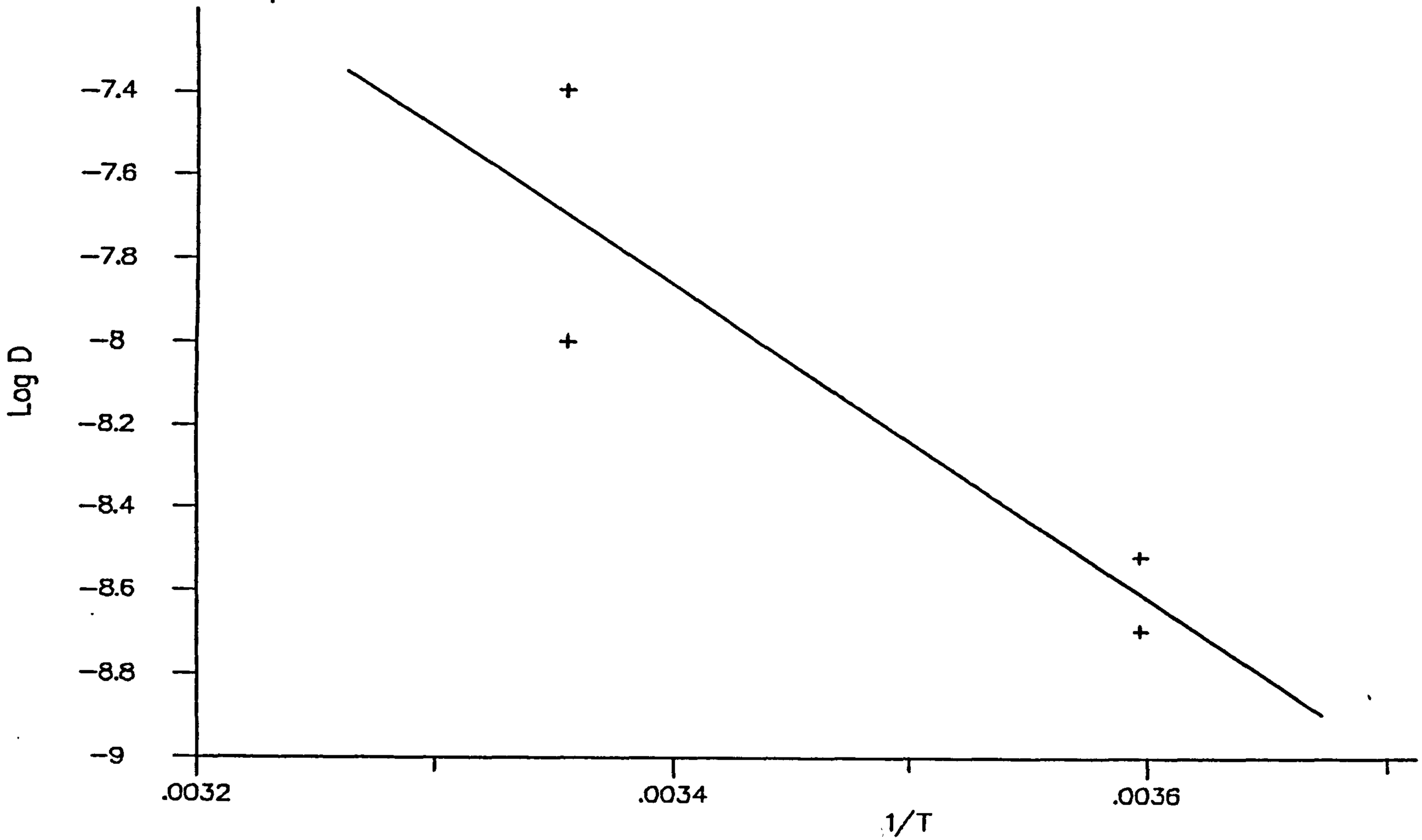
From equation 10 and the values of D given above the activation energy and pre-expontial factor of DEHA in cheese is 3·15 kJmol^{-1} and 1 x 10^{-5} cm^{2}s^{-1} respectively (figure 22). The value of D at any given temperature can therefore be calculated using equation 10.

$$D = D_0 \exp\left(\frac{-\Delta E}{RT}\right) \qquad (10)$$

D - Diffusion coefficient
D_0 - Pre-exponential factor
ΔE - Activation energy
R - Gas constant
T - Temperature in Kelvin

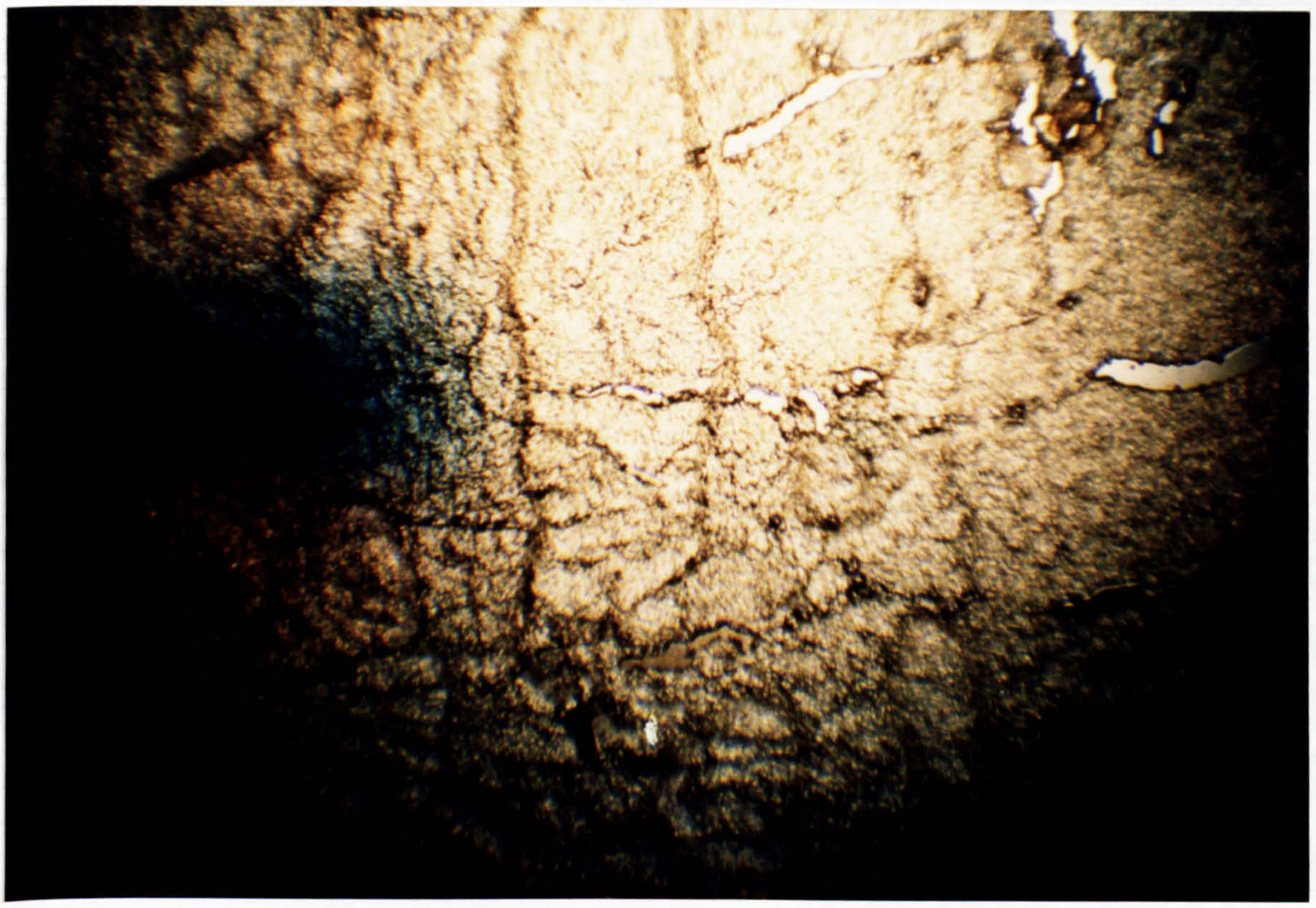
The data points in the region of the concentration profiles corresponding to the doped cheese are more scattered than for the undoped cheese. This may be due to greater structural inhomogeneity of the hand-made doped cheese compared to the commercially produced undoped cheese. Comparison of figure 23 with 24, and 25 with 26, which shows magnified slices of doped and undoped cheese, indicate that the grains of the undoped cheese are smaller and more uniform in size than that of the doped cheese. In addition the number and size of holes (figure 27 is a particularly good example of a hole) in slices of the doped cheese are more variable than for the undoped cheese. This produces imprecision in the calculated DEHA concentration in the cheese which is based on the assumption that each 20μm slice has the same volume and therefore mass.

Figure 22. Plot of the Diffusion Coefficient of DEHA in Cheddar Cheese with Temperature.



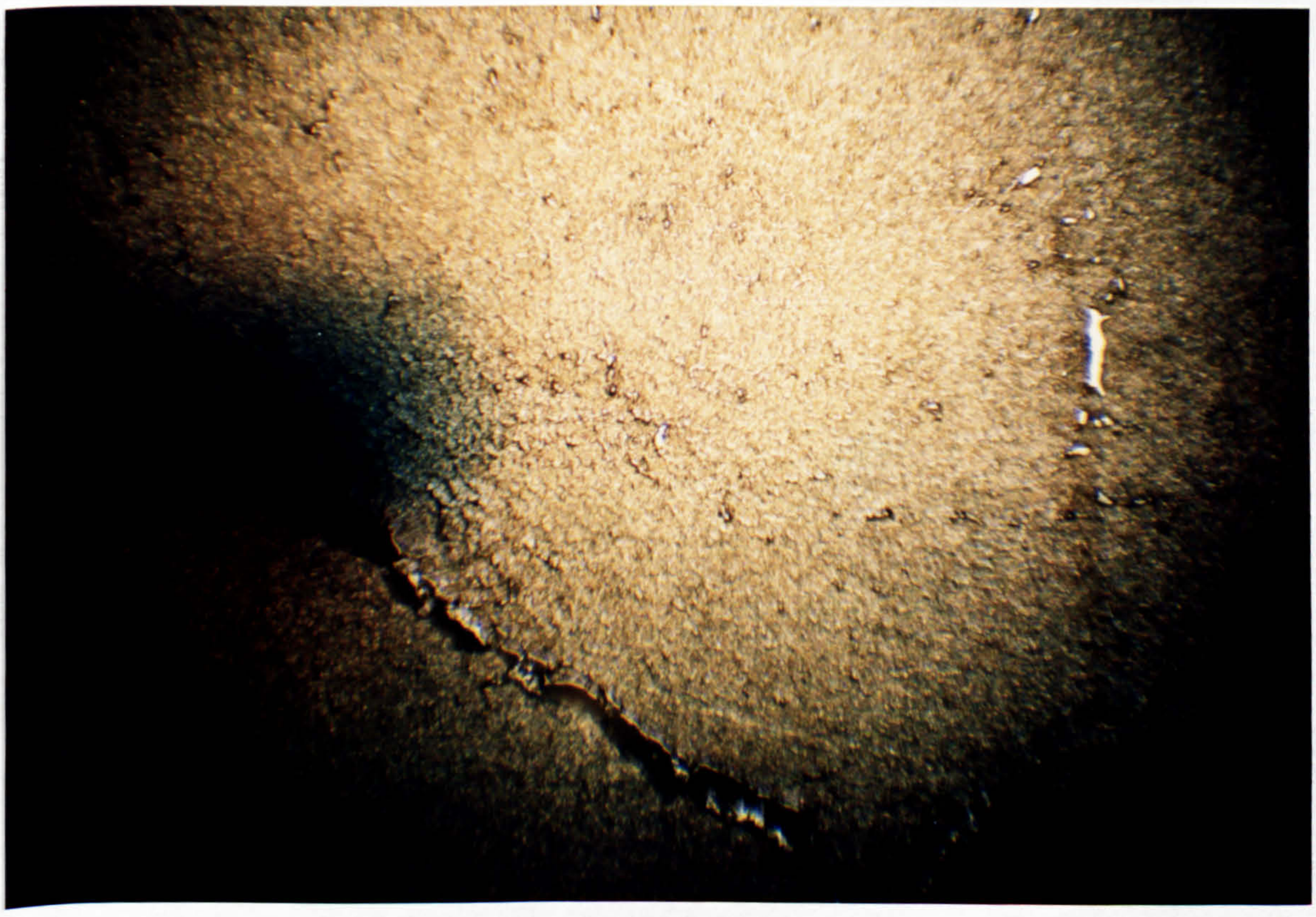
Slope = 3784
Intercept (x = 0) = 5.0

Figure 23. 20µm Section of Cheddar Cheese Doped with DEHA (Magnification × 4).



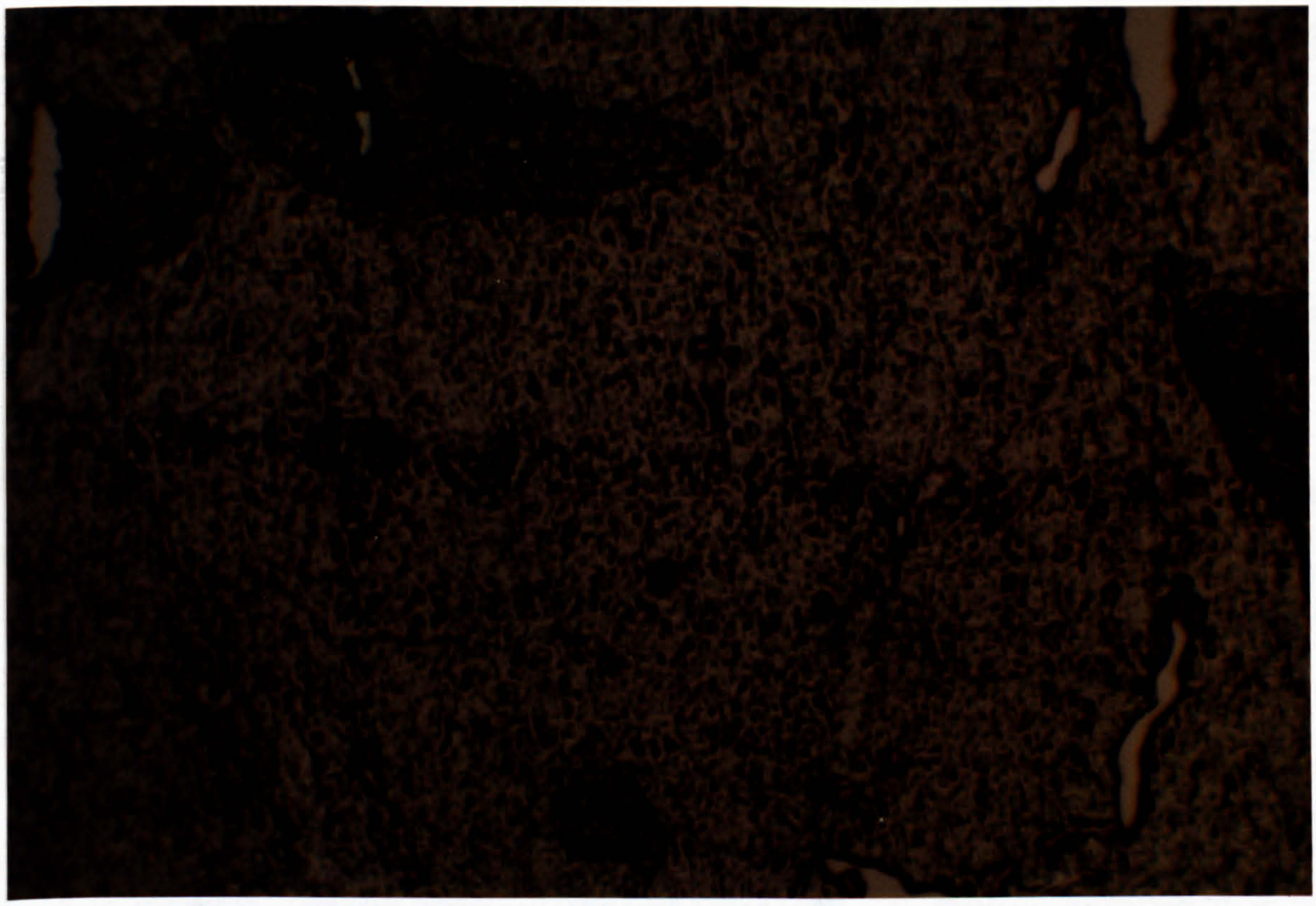
Photograph taken with an OM2 (Olympus) through a Services No. 622104 microscope.

Figure 24. 20µm Section of Cheddar Cheese (Magnification × 4).



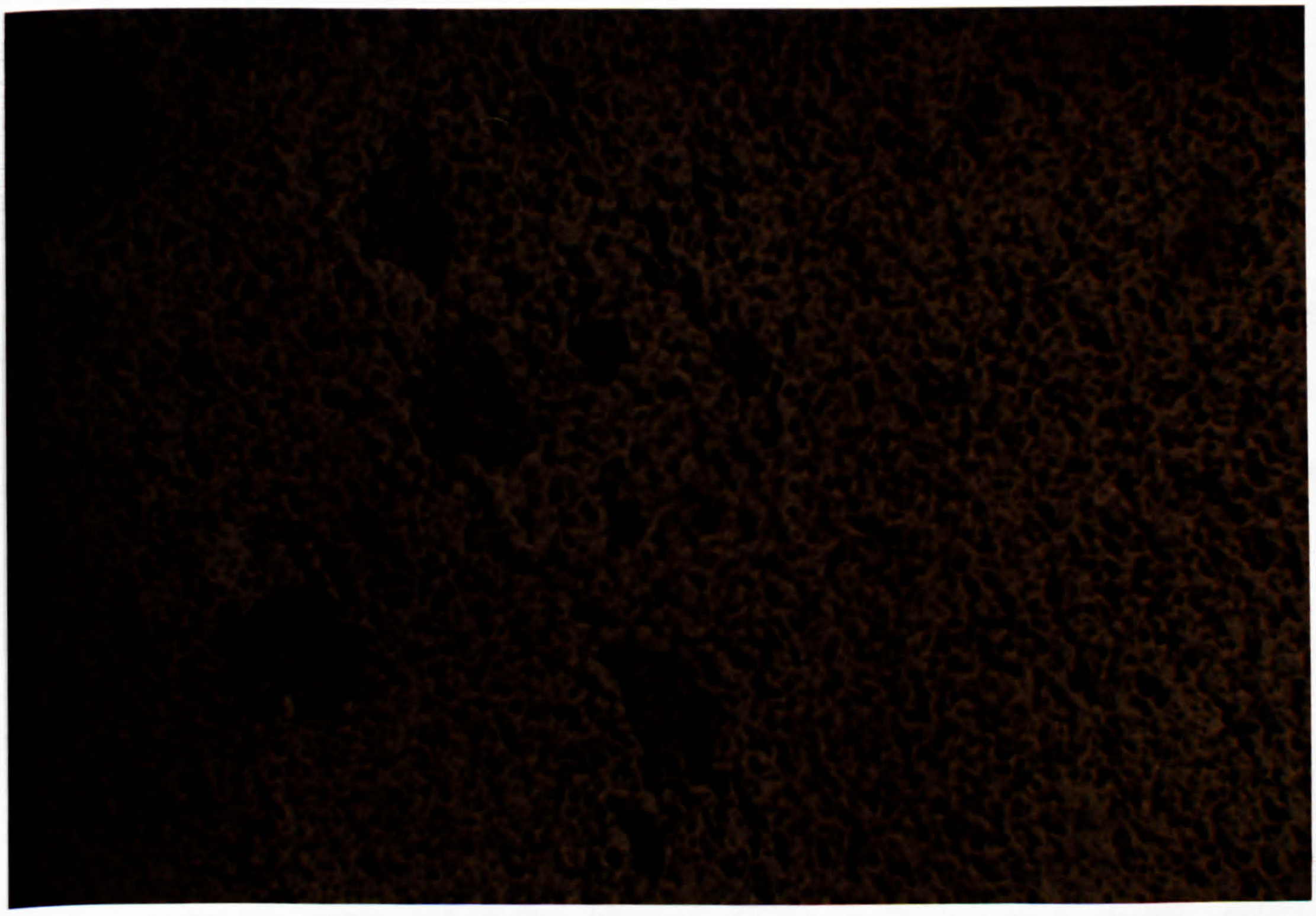
Photograph taken with an OM2 (Olympus) through a Services No. 622104 microscope.

Figure 25. 20µm Section of Cheddar Cheese Doped with DEHA (Magnification × 100).



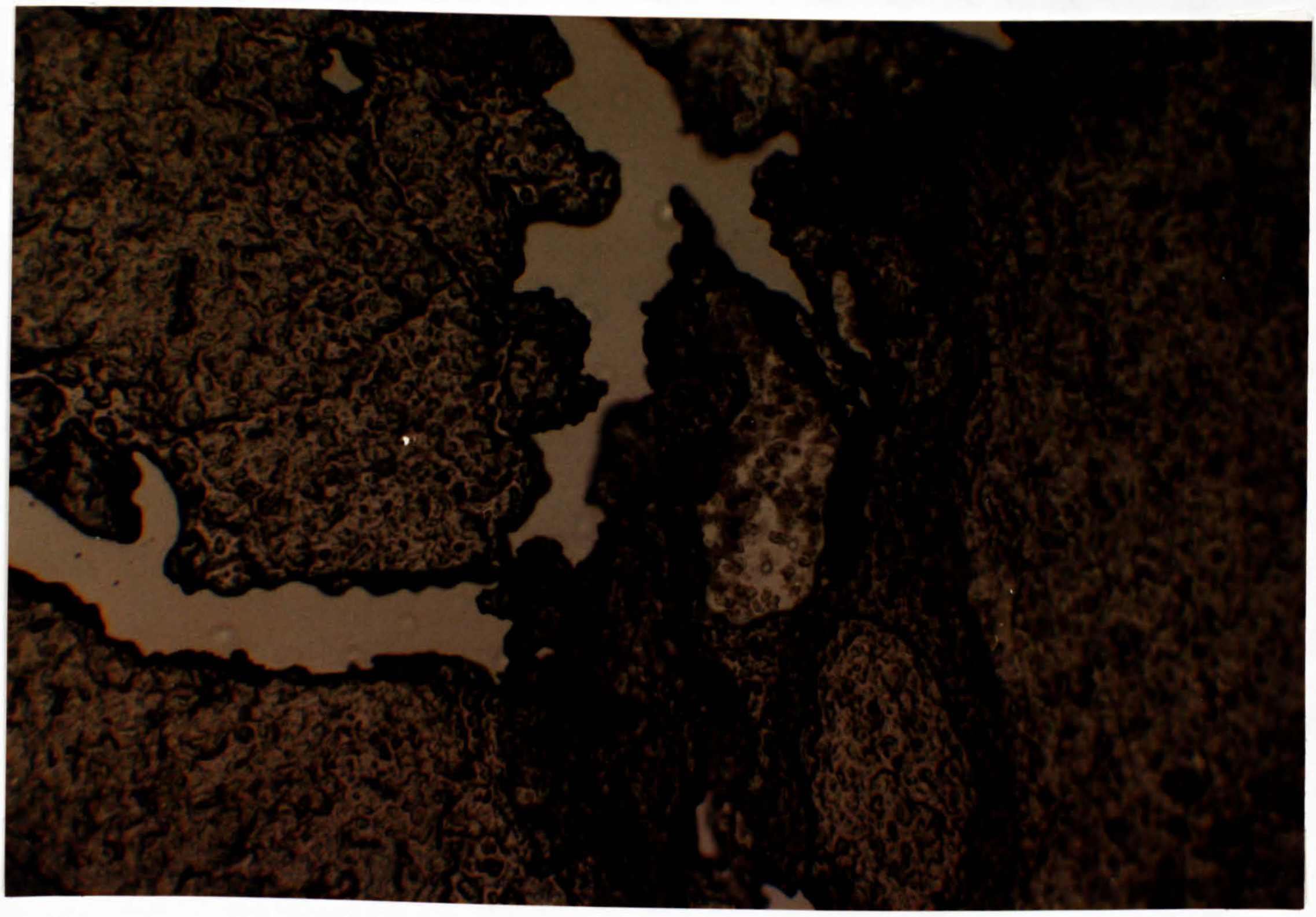
Photograph taken with an OM2 (Olympus) through a Services No. 622104 microscope.

Figure 26. 20µm Section of Cheddar Cheese (Magnification × 100).



Photograph taken with an OM2 (Olympus) through a Services No. 622104 microscope.

Figure 27. An example of a Hole Observed in 20µm Section of DEHA Doped Cheddar Cheese.



Photograph taken with an OM2 (Olympus) through a Services No. 622104 microscope.

4.3.3 DETERMINATION OF THE PARTITION COEFFICIENT OF DEHA BETWEEN CHEDDAR CHEESE AND PVC CLING FILM

DEHA has a low solubility in water and does not partition effectively from ACN into the cheese solid. Therefore the partition coefficient of DEHA between cheese and ACN is effectively that of DEHA between cheese lipid and ACN adjusted for the lipid content of the cheese. The cheese used in this work contained 39% fat, defined as the mass of lipid extracted using acetone/hexane (1:1 v/v).

The partition coefficient of DEHA between Cheddar cheese and cling film (table 14) was calculated using equation 11 and the mean values were 0·58 at 25°C and 0·70 at 5°C. These values were similar to the

value estimated by Till of 0·59. Till estimated the partition coefficient of DEHA between food and PVC to be equal to 1·5 × fraction of fat in the food (19).

$$K_{DEHA\ Cheese/Film} = \frac{K_{DEHA\ Cheese/ACN} \times \%\ Fat}{K_{DEHA\ Film/ACN}} \qquad (11)$$

Table 14. The Partition Coefficient of DEHA.

	5°C			$\bar{x}$	25°C			$\bar{x}$
Cheese lipid/ACN	3·13	2·84	2·98	= 2·98	3·19	3·66		= 3·43
Cling film/ACN	1·61	1·71	1·66	= 1·66	2·08	2·54	2·54	= 2·39
Cheese/Cling film	0·76	0·65	0·70	= 0·70	0·60	0·56		= 0·58

4.3.4 EVALUATION OF TILL'S MATHEMATICAL MODEL OF ADDITIVE MIGRATION

The migration of DEHA into a variety of foods due to the simulated home use of PVC cling film at 5°C, chapter 2, was predicted using Till's model (equation 1) and is compared with measured DEHA migration in table 15.

$$M_t = 2C_{Po}\left(\frac{D_p\ t}{\pi}\right)^{1/2}\ \frac{\beta}{1+\beta} \qquad \beta = K\left(\frac{D_f}{D_p}\right)^{1/2} \qquad (1)$$

M_t - mass of additive migrated from the polymer in time t
C_{Po} - original concentration of the additive in the polymer
t - time
K - partition coefficient of additive between polymer and food
D_f - diffusion coefficient of the additive in the food
D_p - diffusion coefficient of the additive in the polymer

The values used in the prediction of the level of DEHA migration were as follows; C_{P_o} = 0·21 gcm^{-3}; D_p = 2.3 × 10^{-12} cm^2s^{-1} (125); D_f = 1·5 × 10^{-9} cm^2s^{-1} and K = 0·58. The partition coefficient was adjusted, using equation 12, for the fat content of the food for foods other than Cheddar cheese.

$$K_{DEHA\ Food/Film} = K_{DEHA\ Cheese/Film} \times \frac{\%\ Fat\ Food}{\%\ Fat\ Cheese} \qquad (12)$$

Table 15. Comparison of Measured and Predicted Levels of DEHA Migration From PVC Cling Film into a Variety of Foods at 5°C (D_f = 1·5 × 10^{-9} cm^2s^{-1}).

Food Type	Exposure days	% Fat	Measured Migration $mgdm^{-2}$	Predicted Migration $mgdm^{-2}$	Measured Migration / Predicted Migration
Cheddar Cheese	1	39	15·2	10·1	1·5
	5	39	16·8	22·6	0·7
	7	39	19	26·7	0·7
Chicken	7	5	5·4	19·6	0·3
Ham	7	5	2·8	19·6	0·1
Salami	7	45	13·5	26·9	0·5
Lamb	5	18	1·2	21·3	0·1
Beef	5	11	0·9	19.9	0·0
Pork	5	20	5·3	21·5	0·2
Swiss Roll	7	27	8·7	26·1	0·3
Madeira	7	17	2·0	25·0	0·1
Fruit Cake	7	11	0·6	23·5	0·0
Avocado	5	22	11·1	21·7	0·5
Cucumber	5	0·1	<0·1	1·0	0·1

Fat content of food taken from reference 14
The level of DEHA migration was determined in chapter two

The ratios of the measured to predicted level of DEHA migration into Cheddar cheese exposed to PVC cling film for one, five and seven days are 1·5, 0·7 and 0·7 respectively (table 15). The agreement between the measured and predicted levels of migration is particularly good

when viewed in the context of the poor reproducibility of migration experiments into real foods. For example, the level of DEHA migration into triplicate slices of madeira cake taken from the same cake and exposed to cling film for seven days at -18°C (table 4) varied between 0·1 and 0·8 $mgdm^{-2}$.

The agreement between the predicted and measured level of DEHA migration for foods other than Cheddar cheese is reasonable for the high fat content foods, such as salami and avocado where the ratio of the measured to predicted migration is 0·5, but is very poor for the remaining food types, which are low fat content.

The poor agreement between the predicted and measured levels of DEHA migration into low fat content foods may be due to the use of an inappropriate value of D_f. There is evidence in the literature that the diffusion coefficient of DEHA in food varies greatly with food type (125). Till estimated the diffusion coefficient of DEHA in solid food at 4°C was 1×10^{-11} cm^2s^{-1} (125). Recalculation of the level of DEHA migration using this value of D_f gives much better agreement for low fat foods (table 16). For example, the ratios of the predicted to measured level of migration are 1·1, 0·3 and 0·1 (table 16) compared to 0·3, 0·1 and 0·0 (table 15) for swiss roll, madeira cake and fruit cake respectively.

An additional source of error in the prediction of DEHA migration for foods other than Cheddar cheese could be inaccuracy in the value of the partition coefficient used. As discussed earlier, in section 2.3.2., DEHA migration is not proportional to the percentage fat

content of a food. Thus partition coefficients derived from the fat content of the food relative to Cheddar cheese and $K_{DEHA\ cheese/film}$ are prone to errors. However, as Till's model is relatively insensitive to changes in the value of the partition coefficient, a hundred fold change in the value of K produces a five fold change in the level of migration. Errors in the predicted level of DEHA migration due to the use of an inaccurate value of the partition coefficient should be relatively minor.

Table 16. Comparison of Measured and Predicted Levels of DEHA Migration Form PVC Cling Film into a Variety of Foods at 5°C ($D_f = 1 \times 10^{-11}\ cm^2s^{-1}$).

Food Type	Exposure days	% Fat	Measured Migration $mgdm^{-2}$	Predicted Migration $mgdm^{-2}$	Measured Migration / Predicted Migration
Chicken	7	5	5·4	2·5	2·2
Ham	7	5	2·8	2·5	1·1
Salami	7	45	13·5	10·0	1·4
Lamb	5	18	1·2	5·4	0·2
Beef	5	11	0·9	3·9	0·2
Pork	5	20	5·3	5·7	0·9
Swiss Roll	7	27	8·7	8·0	1·1
Madeira	7	17	2·0	6·2	0·3
Fruit Cake	7	11	0·6	4·6	0·1
Avocado	5	22	11·1	6·0	1·9
Cucumber	5	0·1	<0·1	0·05	2·0

Fat content of food taken from reference 14
The level of DEHA migration was determined in chapter two

In summary, the agreement between the measured and predicted levels of DEHA migration from PVC cling film into Cheddar cheese is very good. Reasonable agreement for the high fat content foods is obtained when the partition coefficient used allows for the fat content of the food relative to Cheddar cheese. However, in order to obtain reasonable

agreement for low fat content foods values of the diffusion and partition coefficients other than those measured for Cheddar cheese must be used. Thereby allowing for differences in the composition and nature of the food compared to Cheddar cheese.

Thus Till's mathematical model of additive migration from polymers into food accurately predicts the migration of the plasticiser DEHA from PVC cling film into food and D_f is sensitive to food type.

4.3.4 CLASSIFICATION OF POLYMER/FOOD SYSTEM FOR DEHA PLASTICISED PVC CLING FILM

Polymer/food systems have been classified as either Class I, Class II or Class III (134) in nature.

Class I - there is no migration of additives from the polymer into the food.

Class II - the diffusion coefficient of the additive in the polymer is independent of time and food type in contact with the polymer. The diffusion is Fickian.

Class III - food components penetrate and swell the polymer disturbing its physical structure. Due to this penetration, which is a time dependant process, the diffusion coefficient of the additive in the polymer is dependant on time and food type, and $D_{swollen\ polymer}$ is greater than $D_{polymer}$.

It has been suggested by Chatwin and Katan that most additives in most polymers in contact with fatty foods behave as Class III systems (123). If the migration of DEHA from PVC cling film into fatty foods

is Class III in nature then the value of D_p used in the previous section to predict DEHA migration into food would have been too small.

The migration of DEHA was recalculated using a value of D_p a factor of ten larger than in the previous section. Recalculated levels are compared with the measured migration in table 17. The level of DEHA migration predicted is considerably higher than that measured, the ratios of measured to predicted migration being 0·5, 0·3, 0·3, 0·2 and 0·2 for cheese exposed for one, five and seven days, salami and avocado respectively. Thus by comparison of predicted and measured levels of migration the migration of DEHA from PVC cling film into food is not Class III in nature but is Class II.

A possible criticism of the above argument is that the value of D_p given by Till (125) is not relevant to Class II migration because it was determined by the measurement of the diffusion of DEHA from PVC into corn oil. The corn oil may have penetrated the PVC thereby making the system Class III in nature.

If this was the case then Till's value of D_p is too large for the low fat content foods, such as cooked meats and cakes. To test this hypothesis the level of DEHA migration into these foods was recalculated using a value of D_p a factor of ten smaller than Till's value. The recalculated predicted and measured migration levels are compared in table 17.

Table 17. The Effect of Varying the Diffusion Coefficient of DEHA in PVC (D_p) on the Predicted Level of DEHA Migration from PVC Cling Film into a Variety of Foods at 5°C.

Food Type	Exposure days	Measured Migration mgdm^{-2}	Predicted Migration (mgdm^{-2}) D_p = 10^{-11} cm^{2}s^{-1}	D_p = 10^{-13} cm^{2}s^{-1}
Cheese	1	15·2	28.7	
	5	16·8	64.1	
	7	19	75.8	
Chicken	7	5·4	37.5	7.8
Ham	7	2·8	37.5	7.8
Salami	7	13·5	77.4	
Lamb	5	1·2	54.5	7.1
Beef	5	0·9	46.3	6.7
Pork	5	5·3	56.1	7.2
Swiss Roll	7	8·7	71.1	8.7
Madeira	7	2·0	63.5	8.4
Fruit Cake	7	0·6	54.8	8.0
Avocado	5	11·1	57.4	
Cucumber	5	<0·1	1.1	0.06

Fat content of food taken from reference 14
The level of DEHA migration was determined in chapter two

The agreement between the predicted and measured levels of DEHA migration for low fat foods is poor, the predicted migration being, on average, a factor of three greater than the measured migration.

In addition, the levels of migration predicted is insensitive to food type, with the exception of cucumber, which is contrary to the measured migration. The predicted migration for foods exposed for five or seven days ranged from 6·7-7·2 mgdm^{-2} and 7·8-8·7 mgdm^{-2} respectively compared to 0·9-5·3 mgdm^{-2} and 0·6-8·7 mgdm^{-2} for the measured migration. The insensitivity to food type is because as the value of D_p increases the second term in Till's model, $(D_p t/n)^{½}$

increases in importance and the time of exposure, rather than K or D_f, determines the level of migration of DEHA.

Therefore from the evidence presented above the migration of DEHA from PVC cling film into both high and low fat content foods is Class II in nature.

CHAPTER 5

THE DETERMINATION OF ADIPATE BASED POLYMERIC PLASTICISERS IN FOOD

5.1 INTRODUCTION

Chapters 2 and 3 described the determination of the levels of plasticisers in food resulting from the use of flexible packaging films in the home and for retail goods. These data were employed by the MAFF to calculate maximum dietary values of each of the plasticisers for the UK population. The estimated maximum calculated dietary intake of DEHA was much greater than that of any other plasticiser at 16 mg/person/day compared to 2 mg/person/day for the next highest level (DBP) (117). Although, it was considered that "the possibility of risk to public health from the current estimates of maximum daily intake is remote" (117), the Department of Health Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment recommended that, due to uncertainties in the toxicological data, the dietary intake of DEHA should be substantially reduced as a matter of prudence (117).

The high dietary intake level of DEHA was due to the extensive use of DEHA plasticised films coupled with a relatively facile migration of DEHA from PVC films. DEHA plasticised cling film was the only plasticised PVC packaging material available for domestic use and was the most extensively used plasticised material for retail packaging. In order to reduce the dietary intake of DEHA the film manufacturers investigated alternative plasticisers for PVC cling film which would not migrate into food to the same extent as DEHA.

There are two possible approaches in designing plasticisers with low migration characteristics. The first is to chemically bond the

plasticiser to the polymer. The second is to increase the molecular size of the plasticiser and thus restrict its movement through the polymer. An example of the second approach, which was the one favoured by industry, was the use of the so-called "polymeric" plasticisers. A typical example of these high molecular weight plasticisers is Reoplex 346 (R346, Ciba Geigy) which is a copolymer of 1,3-butandiol and adipic acid with a number-average molecular weight of 1950 daltons compared to that of DEHA at 370 daltons.

Reoplex 346

$$YOOC-(CH_2)_4-COO-[X-OOC(CH_2)_4-COO]_n-Y$$

$X = OCH_2CH_2CH_2(CH_3)O$ Y= adipic acid or X

A method of analysis for adipate-based polymeric plasticisers in food was developed so that data on their migration characteristics could be generated for the eventuality that polymeric plasticisers should prove to be commercially viable. R346 was chosen as a typical polymeric plasticiser for method development purposes.

For reasons of sensitivity and selectivity, the analytical method of choice is GC-MS, but the high molecular weight of polymeric plasticisers precludes direct analysis on current GC columns and the polydisperse nature of the plasticiser (polymerics plasticisers are oligomer mixtures) prevents determination as a single analyte. This means that any detector signal is spilt between the oligomers present and low limits of detection are therefore difficult to achieve.

Two methods of determining migration levels of polymeric plasticisers into food were available from the literature. The first involved exposing food to the plasticised film and measuring migration as weight loss from the film (133). This method was unsuitable as the anticipated losses from the film were low, about 1% by mass, and thus the weight difference would be small and difficult to detect with a high degree of precision or accuracy. Furthermore, it was likely that the loss of plasticiser from the film would be masked by the migration of food lipids into the film. The second method involved extraction of the plasticiser from the food, transmethylation of the extracted fat, and analysis as dimethyl adipate (DMA) by GC-FID using dimethyl pimelate (DMPim) as an internal standard. However the method had only been applied to the analysis of spiked fat samples and had a detection limit of 8·3 $mgkg^{-1}$ (134) which it was anticipated would be inadequate for this work. The second method was taken as the basis for a stable isotope dilution technique who's development is described here. The technique was then successfully applied to the analysis of polymer plasticiser in a variety of food types, see chapter six.

5.2 EXPERIMENTAL

5.2.1 MATERIALS

5.2.1.1 Primary and Internal Standards

The following primary standards were available commercially; DCHP (British Cellophane Ltd.), DEHA (Hexaplas DOA, ICI), DMA and DMPim (Aldrich Chemical Company), R346 (Ciba-Geigy). d_4-DEHA was available

from earlier work (104). All standards, except R346 which was not amenable to GC analysis, were better than 96% pure when analysed by capillary GC.

5.2.1.2 Reagents and Solvents

Boron trifluoride etherate (BF_3 etherate, Sigma Chemical Company), butan-1-ol, and hexan-1-ol (BDH Chemical Company) were available commercially. All solvents were of HPLC-grade and supplied by Rathburn (Walkerburn, Scotland) unless stated otherwise.

5.2.2 ANALYTICAL METHOD

5.2.2.1 Extraction

Samples were weighed accurately and those which weighed more than 50g were homogenized in a domestic food processor (Type 4243, Braun) and a sub-sample (30g) taken. The sub-sample was then homogenized (Ultra-Turrax) in acetone/hexane (1:1 v/v, 100 mL), after the addition of d_4-DEHA internal standard (1·0-0·1 mg according to food type). The homogenized mixture was then set aside for one hour to allow the internal standard and analyte to equilibrate. The supernatant was decanted from the homogenate which was then extracted with a further portion of solvent (100 mL). The combined extracts were dried (Na_2SO_4) and the solvent removed under reduced pressure. The weight of the residue was noted to give an indication of the fat content of the sample.

5.2.2.2 Transmethylation

A portion of the extracted fat sample (0.5g) was reacted with BF_3 etherate in methanol (MeOH) (12% v/v, 4 mL) for two hours at 60°C with

shaking every 30 minutes. The reaction was then quenched with distilled water (3 mL) and the deuterated and non-deuterated dimethyl adipate (d_4-DMA, d_0-DMA) extracted into DCM/C_6H_{12} (3:7 v/v, 9 mL). The bottom aqueous methanol layer, containing dissolved boron salts, was removed and the remaining organic phase dried (Na_2SO_4). This was then decanted from the drying agent and evaporated to a small volume (ca. 2 mL) under reduced pressure and made up to 4 mL with cyclohexane in readiness for clean up by SEC.

5.2.2.3 Clean up

The d_0-DMA and d_4-DMA were separated from the methylated lipids present by SEC using the automated system described in section 2.2.2.1. The appropriate collection window for d_0-DMA and d_4-DMA (81-88 minutes) was determined according to section 5.2.3.6, and this fraction was collected and evaporated under reduced pressure to a small volume (ca. 1 mL) prior to analysis by SIM GC-MS. The SEC eluant was monitored at 254 nm and DCHP, as a UV-active retention marker, interspersed with the samples to monitor the flow rate.

5.2.2.4 SIM GC-MS Analysis

The SIM GC-MS analysis was carried out on the system described in section 2.2.2.2. The column was held isothermally at 110°C, the injection split 20:1 and the mass spectrometer operated at a resolution of 500 (10% valley definition). The ions at m/z = 143 and 101 (DMA) and 147 and 103 (d_4-DMA) were followed with dwell times of 100 ms per mass and a setting time of 20 ms to give a total cycle time of 420 ms. Quantification was based on standard calibration curves for R346/d_4-DEHA put through the full method at known weight ratios.

5.2.3 METHOD VALIDATION

5.2.3.1 Equilibrium Time for Extraction of R346 and d_4-DEHA

A slice of cheddar cheese (60 x 85 x 4 mm) was wrapped completely in polymerically plasticised cling film and stored for one day at room temperature. The sample was then unwrapped, added to acetone/hexane (1:1 v/v, 100 mL) containing d_4-DEHA (1 mg) and left to stand for one hour. It was then homogenized (Ultra-Turrax) and a 20 mL aliquot of solvent removed. Thereafter, at various time intervals the sample was homogenized and further 20 mL aliquots of solvent removed. The system was kept at constant volume throughout the experiment by the addition of replenishing solvent. The 20 mL aliquots were evaporated to a small volume (ca. 1 mL) and the ratio of R346 to d_4-DEHA determined by SIM GC-MS analysis as outlined in section 5.2.2.4.

5.2.3.2 Quantitative Extraction of R346 and d_4-DEHA from Food

A solution of R346 (5 mg) and d_4-DEHA (6 mg) in diethyl ether was added to the surface of a slice of cheddar cheese and left to stand for one hour. The sample was then repeatedly homogenized (Ultra-Turrax) with portions of acetone/hexane (1:1 v/v, 100 mL). On each occasion the supernatant was decanted, and analysed for R346 and d_4-DEHA as outlined in section 5.2.2.

5.2.3.3 Selection of Transesterfication Alcohol for R346 Breakdown

Solutions of BF_3 etherate (15% v/v) in butan-1-ol, hexan-1-ol, 2-ethylhexan-1-ol or methanol were prepared. These solutions were then used in the transmethylation step of the procedure given in section 5.2.2.2 in the analysis of R346 standards.

5.2.3.4 Reaction Time for Transmethylation of R346

R346 (20 mg) and DMPim (26 mg, internal standard) were reacted with BF_3/MeOH (12% v/v, 1 mL) at 60°C. At various time intervals a 50µL aliquot was removed and quenched as in section 5.2.2.2. The samples were then analysed by GC using the system described in section 3.2.2.1 and the following column temperature programme; 120°C for 2 mins. then 20°Cmin-1 to 300°C.

5.2.3.5 Preferential Loss of Internal Standard on Evaporation

Cheddar cheese fat (10g) was spiked with d_0-DEHA (3 mg) and d_4-DEHA (3 mg). The sample was then extracted, transmethylated, quenched and the d_0-DMA and d_4-DMA extracted into DCM/C_6H_{12} as in section 5.2.2.2. Aliquots (10 mL) of the sample were then taken, DMPim (0·28 mg) added as an internal standard, and evaporated under reduced pressure to various volumes including to dryness. The d_0-DMA/d_4-DMA ratio in each of the samples was then measured by SIM GC-MS as described in section 5.2.2.4.

5.2.3.6 Determination of SEC Elution Time of DMA

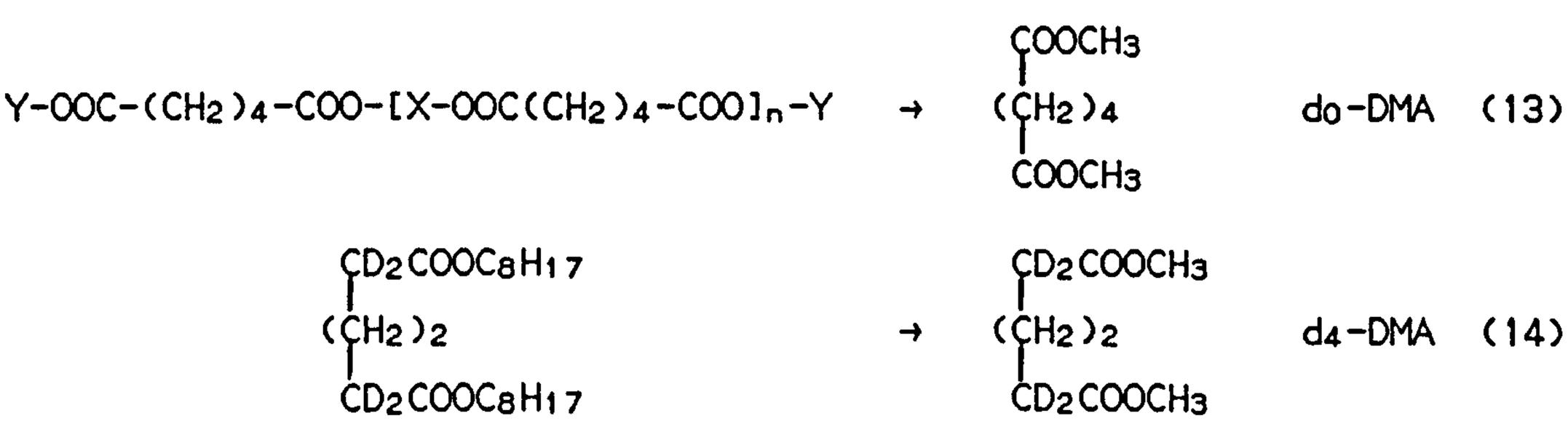
A standard of DMA (2 mg) and DMPim (2 mg, internal standard) was loaded onto the automated SEC system outlined in section 2.2.2.1 and three minute fractions of the eluant collected from 65 minutes onwards. The fractions were then analysed by GC using the system described in section 3.2.2.1 with the column held isothermally at 120°C.

5.3 RESULTS AND DISCUSSION

5.3.1 METHOD

5.3.1.1 Overview of Method

As with the analysis for DEHA (section 2.3.1) and certain of the phthalate plasticisers (section 3.3.1.3) the analysis of polymeric plasticisers in food used a stable isotope dilution technique with its inherent advantages. The plasticiser and internal standard were extracted from the homogenized food sample, together with food lipids. Transmethylation was used to then convert all adipate-based plasticisers present to d_0-DMA and the deuterated internal standard to d_4-DMA (equations 13 and 14). Thereafter, the method was identical in principle to that for DEHA.



X = $OCH_2CH_2CH_2(CH_3)O$ Y = adipic acid or X

5.3.1.2 Analytical Methodology

The SIM GC-MS chromatograms of contaminated food samples showed smooth clean peaks for both d_0-DMA and d_4-DMA. The retention times for the samples and standards were essentially identical as can be seen in

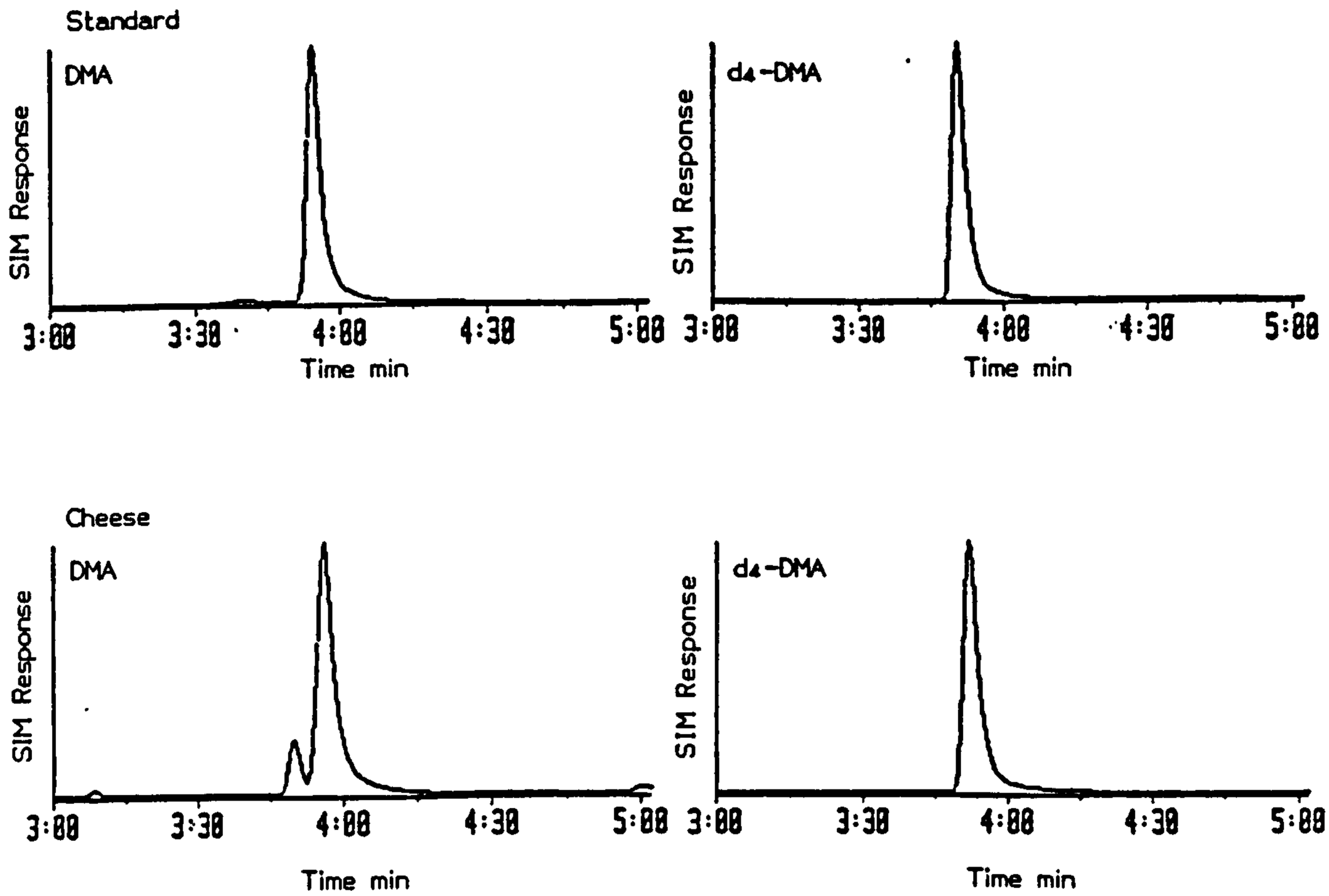
figure 28 which shows some typical traces. Either channels m/z = 143 and 147 or 101/103 (d_0-DMA and d_4-DMA) could be used for quantification. The calibration curve obtained for standards was linear, figure 29, with a correlation coefficient of 0.9998 and a standard error of 0.0890. There were no interfering peaks in the SIM GC-MS chromatograms of the reagent and sample blanks. The use of a stable isotope internal standard compensates for any losses during sample preparation and analysis. The RSD for the method was 3% which compares favourably with the RSD of between 22% and 55% for replicate migration experiments (section 6.4.2).

5.3.1.3 Validation Experiments

All stages of the method were validated for the analysis of R346, the results of the validation experiments are discussed below.

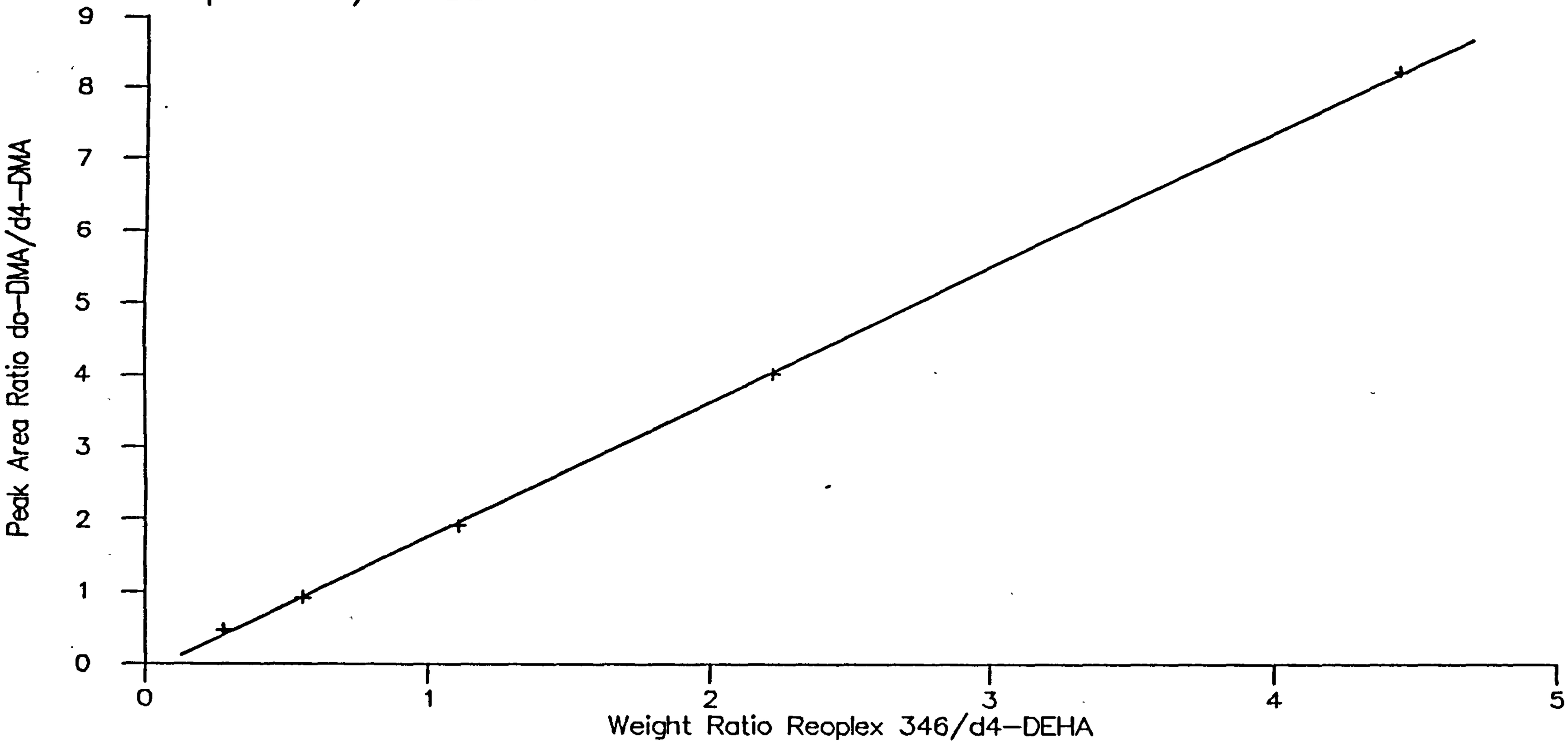
It can be seen from figure 30 that R346 and d_4-DEHA come to equilibrium in the homogenized food sample within one hour. They can then be quantitatively extracted with two portions of solvent (acetone/hexane 1:1 v/v, 100 mL x 2), figure 31. Acetone/hexane (1:1 v/v) was selected as the extractant as it had been successfully used to extract DEHA from various foodstuffs (section 2.2.4 & 3.2.3.2) and there was evidence in the literature to suggest that polymeric plasticisers are more readily extracted than DEHA (134).

Figure 28. SIM GC-MS Chromatograms for the Analysis of R346 in Cheese contaminated at 12 mgkg^{-1}.



SIM GC-MS conditions: 30m x 0·24 mm I.D. DB5 fused silica column operated at a helium carrier gas flow rate of 1mLmin^{-1} at an isothermal column temperature of 110°C. Ions monitored were m/z = 143 d_0-DMA and m/z = 147 d_4-DMA (internal standard). Dwell times of 100ms and setting times of 20ms gave a total cycle time of 420ms.

Figure 29. Calibration Graph for the Analysis of Reoplex 346, as DMA, by SIM GC–MS. Plot of Peak Area Ratio of do–DMA/d4–DMA versus Weight Ratio of Reoplex 346/d4–DEHA.



Slope = 1.87
Correlation Coefficient = 0.9999
Intercept (x = 0) = –0.12

Figure 30. Determination of the Time required for Cheddar Cheese spiked with R346 and d4-DEHA in acetone/hexane (1:1 v/v) to come to Equilibrium.

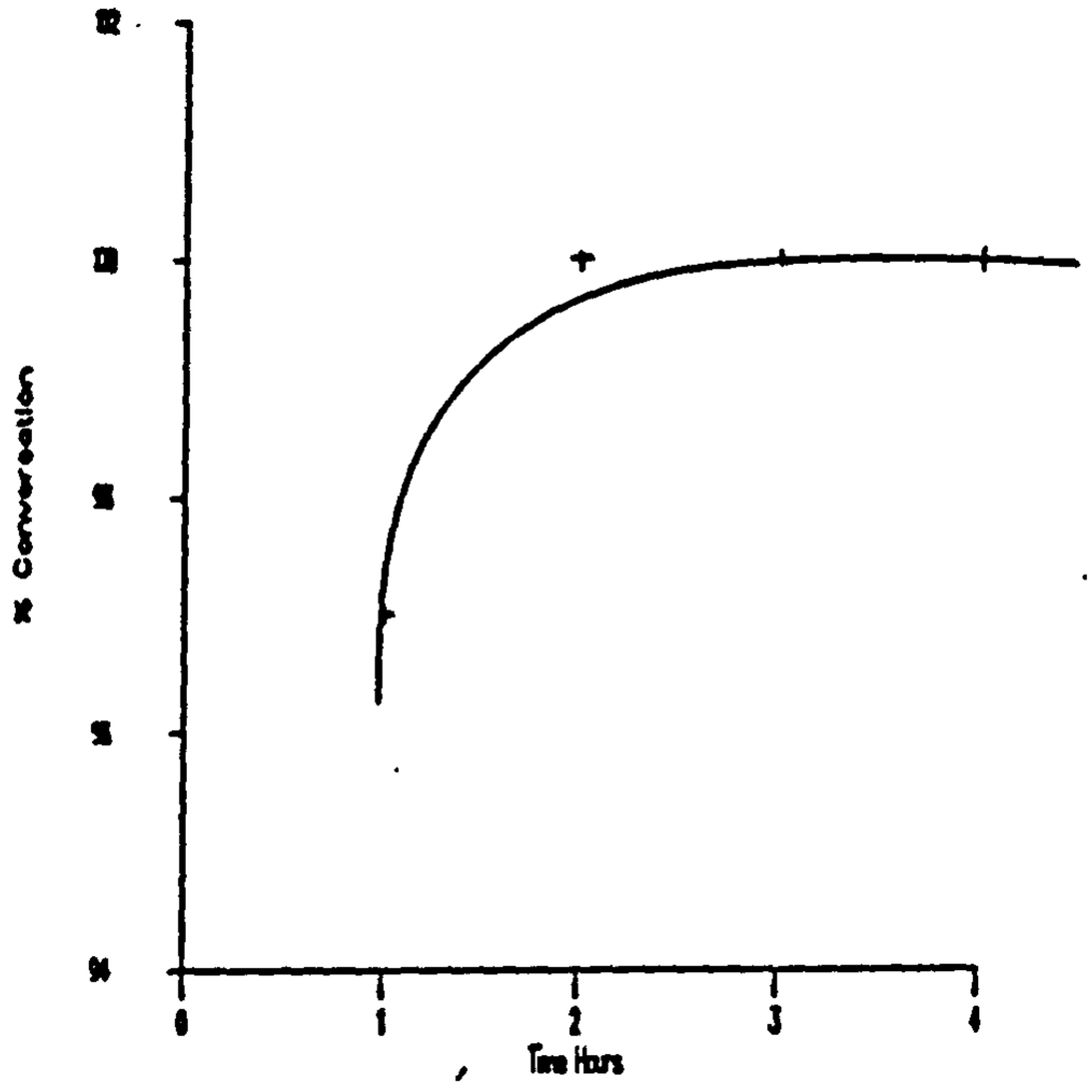
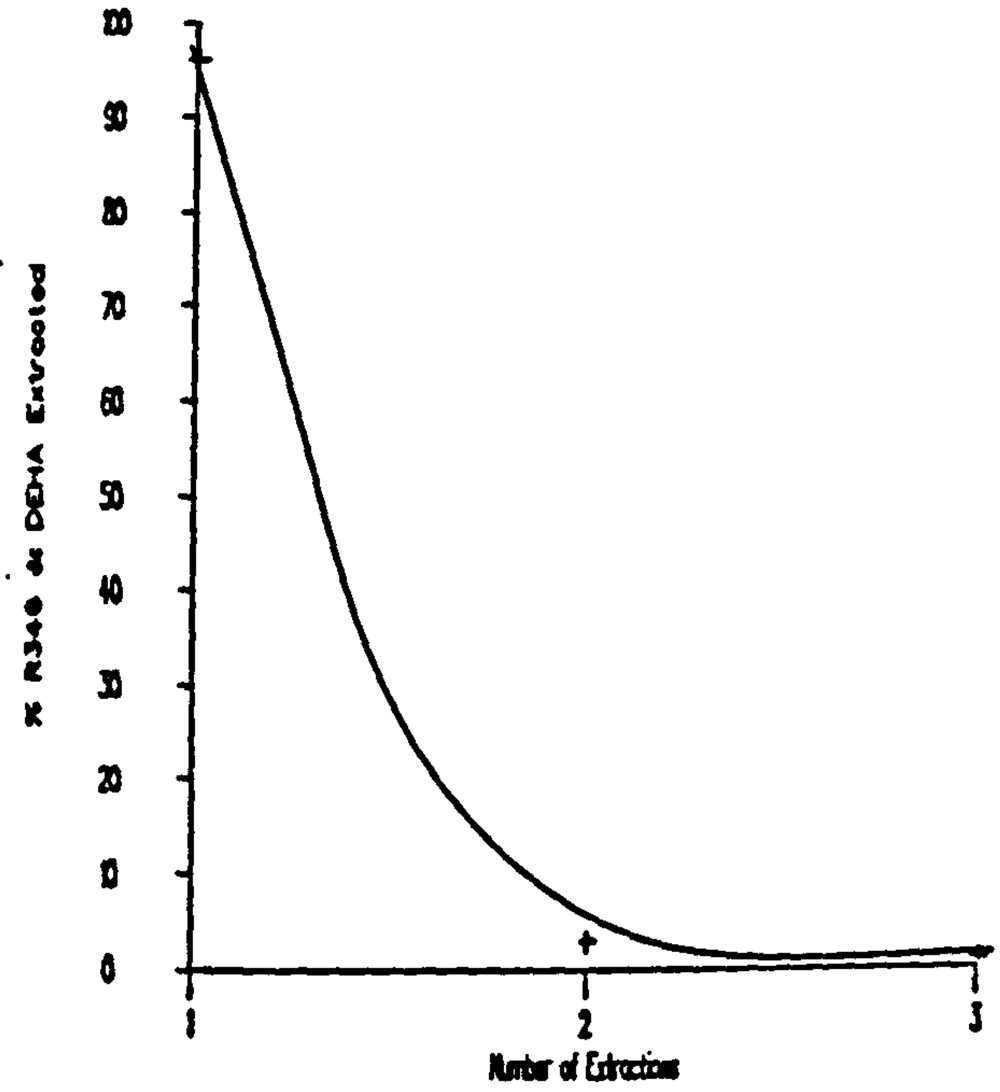


Figure 31. Determination of the Solvent Volume required to Quantitatively Extract R346 and DEHA from Cheddar Cheese.



Each extraction used 100 mL of acetone/hexane (1:1 v/v)

Figure 32. Determination of the Transmethylation Reaction Time for R346.

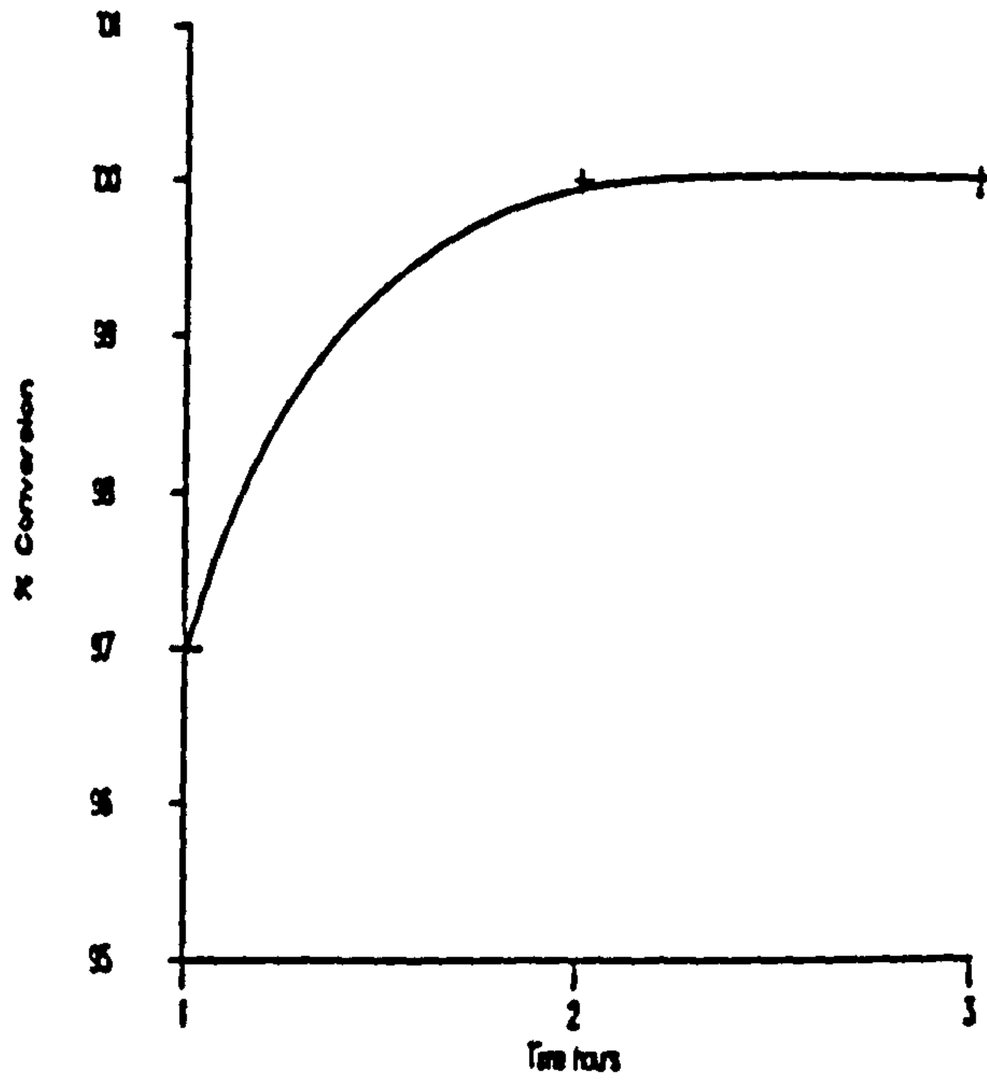
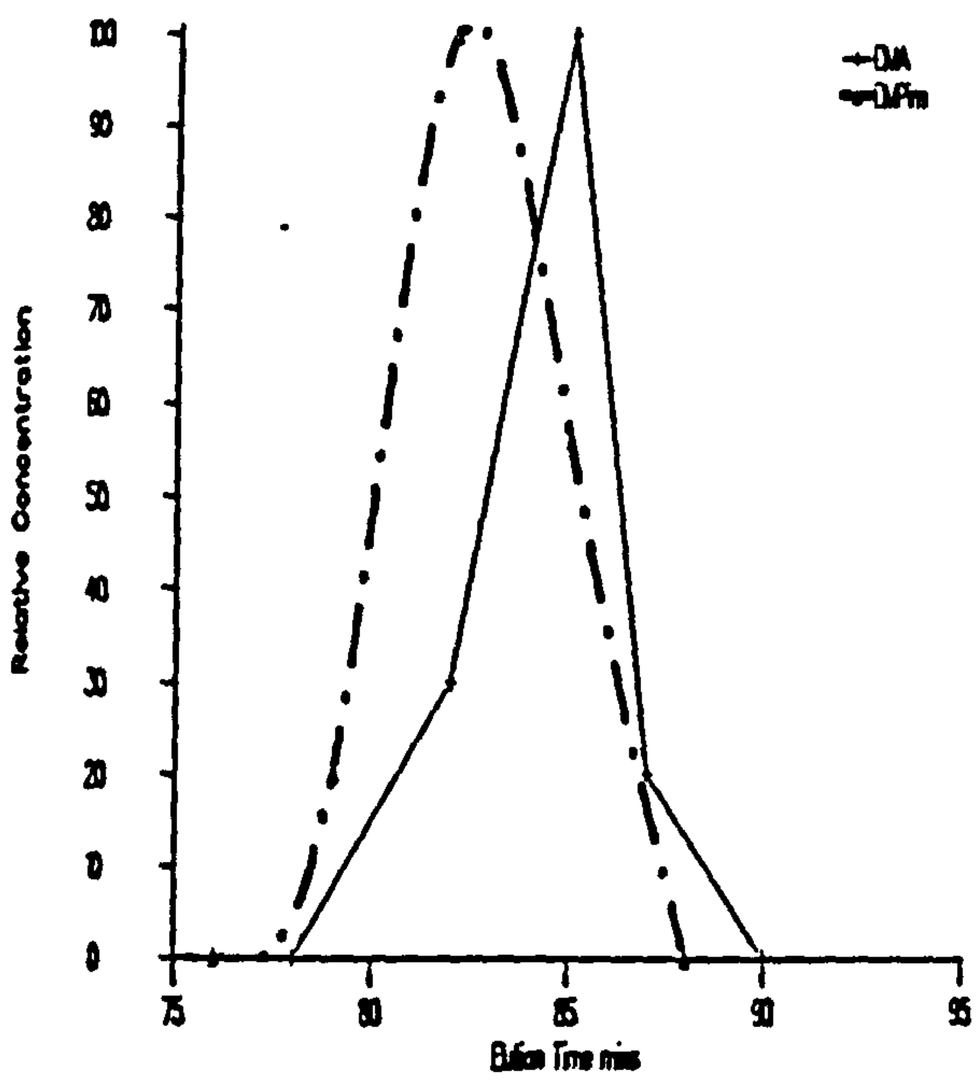


Figure 33. The OCSEC Elution Profile of DMA and DMPm.



A variety of alcohols (butan-1-ol, hexan-1-ol, 2-ethylhexan-1-ol or methanol) were use to transesterfy R346 to a single analyte. Transmethylation was selected because the reaction came to completion more rapidly than the transesterfication with the higher alcohols. This was due to the lower molarity of the higher alcohols, the alcohol being used as reagent and reaction solvent.

Transmethylation was carried out at 60°C, which being 5°C below the boiling point of methanol allowed the reaction to be performed in septum-capped vials in a minimum amount of time. From Figure 32 it can be seen that the reaction comes to completeness within 2 hours. The ratio of d_0-DMA to d_4-DMA remained constant, as determined by GC-MS, during evaporation of solutions under reduced by pressure indicating that no preferential loss occurs.

Figure 33 shows the SEC elution profile of DMA and DMPim thus establishing the elution time of DMA and thus the appropriate collection window for d_0-DMA and d_4-DMA. In addition, the virtual co-elution of DMA and DMPim illustrates, with regard to SEC, the suitability of DMPim as an internal standard where the end determination is by GC rather then GC-MS.

To ensure that there was no discrimination against d_0-DMA or d_4-DMA on SEC, a sample of d_4-DMA and d_0-DMA, in a known ratio, was injected on to the SEC column and the appropriate fraction collected and analysed by GC-MS. The ratio of d_4-DMA to d_0-DMA was not affected by the SEC proving that, as anticipated, the two compounds co-elute.

5.3.2 APPLICATIONS

5.3.2.1 Foodstuffs

The migration levels of R346 from PVC cling film into cheese, cake, sandwiches, chicken and peanut biscuits have been determined (chapter 6) without any interference problems. As these foods vary in fat, sugar and moisture content, this demonstrates that the method given in section 5.2 is applicable to the determination of any polymeric adipate plasticiser contamination in a wide variety of food types.

5.3.2.2 Film Types

As discussed in section 6.1 two approaches to formulating films plasticised with polymeric plasticisers have been adopted. To use polymeric plasticisers either alone or in conjunction with DEHA. Since the analytical method given in section 5.2 measures the total adipate level in the food, if the level of polymer plasticiser contamination from a mixed plasticiser film is to be determined then the level of DEHA, or any other monomeric adipate ester, must be determined independently. The level of polymeric plasticiser present is then equal to total adipate, as determined as DMA, minus that yield of DMA attributable to the monomeric adipate esters. For this correction to be successful the levels of DEHA, for example, determined as DMA must correspond exactly with the levels as determined as DEHA itself. If, for example, DEHA is partially hydrolysed in the foodstuffs and yields more DMA than the DEHA level would suggest, then the value obtained for polymer plasticiser contamination would be incorrect (too high). Results of experiments carried out (chapter 6) indicate this is not the case and that it is possible to analyse for polymer plasticiser in the presence of DEHA.

It may also be possible to analyse for polymeric plasticiser in the presence of other monomeric adipate-based plasticisers providing that control experiments are conducted to ensure there is no interference from the other plasticiser(s) present.

5.3.2.3 Polymeric and Isomeric Plasticisers

In principle the method of analysis reported in section 5.2 is suitable for the analysis of any polymeric or mixed-isomeric ester plasticiser. Those of greatest interest would be plasticisers based on phthalic acid. Clearly it would not be prudent to use this method without first carrying out control experiments similar to those carried out for the analysis of R346.

CHAPTER 6

COMPARATIVE STUDY OF THE MIGRATION OF CONVENTIONAL (DEHA) AND POLYMERIC (REOPLEX 346) PLASTICISERS FROM PVC CLING FILM INTO FOOD

6.1 INTRODUCTION

As indicated in section 5.1, PVC film manufacturers were investigating the use of polymeric plasticisers for the production of PVC cling films with lower migration characteristics than DEHA plasticised cling film. In order to assess the migration characteristics of polymeric plasticised cling film a limited series of migration experiments was carried out to determine the level of polymeric plasticiser migration into food.

Two approaches to film formulation were being adopted, the partial or total replacement of DEHA with polymeric plasticiser. Thus three films were studied; conventional cling film (DEHA alone), mixed plasticiser cling film (DEHA and R346) and polymeric plasticiser cling film (R346 alone). The migration experiments were carried out under identical conditions for all three films so that the results obtained would be readily comparable. Conventional cling film was included in the study to give an indication of the relative ease of migration of R346 to DEHA. The migration experiments conducted were those which had given the maximum migration levels in previous studies (chapter 2, 11) and thus represented a severe test of the alternative films.

6.2 EXPERIMENTAL

6.2.1 MATERIALS

6.2.1.1 Primary and Internal Standards, Reagents and Solvents

DMA (Aldrich Chemical Company) and R346 (Ciba-Geigy) were commercially available and d_4-DEHA was available in the laboratory from earlier work (104).

The reagent used for transmethylation, BF_3/MeOH complex ca. 12% w/w BF_3 was commercially available (Aldrich Chemical Company). All solvents were of HPLC-grade and supplied by Rathburn (Walkerburn, Scotland) unless stated otherwise.

6.2.1.2 Films and Food

The following PVC films were used; conventional cling film, 17% w/w DEHA, (Borden Ltd.); mixed plasticiser cling film, 10% DEHA w/w and 10% w/w R346, (Filmco International Ltd.) and polymeric cling film, 23% w/w R346, (Filmco International Ltd.). The type and level of each plasticiser present was confirmed by analysis.

Foodstuffs were purchased from local retail outlets where it had been established that the products had not previously been in contact with adipate-based plasticised film. This was confirmed by the analysis of blanks.

6.2.2 MIGRATION EXPERIMENTS

Three replicate migration experiments were carried out for each film and food-type combination. The experiments were conducted in an as reproducible a manner as possible, effort was made to ensure that the film was uniformly and reproducibly stretched when wrapping samples. The total and sub-sample weights, total amount of film used, and area of film in contact with the sample were recorded.

6.2.2.1 Migration into Cheese Slices

A glass plate (ca. 20 x 20 cm) was covered with the appropriate film and three cheese slices (J. Sainsbury, natural cheddar, ca. 8·2 x 10·0 cm each) placed on it. A second film-covered plate was then placed on top of the cheese to give a film/cheese/film sandwich. The glass plates were covered with film as uniformly as possible to ensure good reproducibility of the cheese-to-film contact. The samples were stored for 5 days at 5°C.

6.2.2.2 Migration into Swiss Roll

Complete Swiss Rolls (J. Sainsbury, Chocolate Cream) were wrapped in film with a minimum amount of overwrapping and stored for 5 days at 5°C.

6.2.2.3 Migration into Egg Mayonnaise Sandwiches

Sandwiches, in rigid non-plasticised plastic boxes, were purchased from a local sandwich bar. They were unboxed, wrapped in film, and stored for 1 day at 5°C.

6.2.2.4 Migration into Peanut Biscuits and Chicken Breasts during Cooking in a Microwave Oven

These cooked dishes were prepared according to published recipes (135) which involved cooking the samples in contact with film, in a microwave oven. The chicken breasts were dotted with butter and overwrapped on a shallow plate with film. They were then cooked for 3 minutes on high power (600W). The cooking tray for the peanut biscuits was lined with film and the sample cooked on high power for 4 minutes.

6.2.3 ANALYSIS

The level of DEHA migration from conventional cling film into food was determined using the method given in section 2.2.4. The level of R346 migration from polymeric cling film into food was determined using the method given in the previous chapter. The level of DEHA and R346 which migrated into food from the mixed plasticiser cling film was determined by the analysis of separate sub-samples for either DEHA or R346 using the methods given above.

6.3 RESULTS AND DISCUSSION

6.3.1 ANALYTICAL METHODOLOGY

The analytical methods utilised for this work have previously been discussed (DEHA section 2.3.1, R346 section 5.3.1). The accuracy and precision of the methods are good, with a relative standard deviation of 2-3% for the analysis of replicate sub-samples.

6.3.2 MIGRATION DATA

The aim of this section of work was to determine the level of R346 migration into a variety of food types. The average migration levels for the three films have been summarised in table 18 for comparison. As the migration experiments for all three films were carried out under identical conditions, as far as is practically possible, and the films used had approximately the same thickness and weight per unit area, the results obtained should reflect the relative ease of migration of each plasticiser. Each film type is discussed individually below.

6.3.2.1 Conventional Cling film.

Migration levels of DEHA from conventional cling film (table 19) were consistent with those reported in the literature (106). Levels of between 37-362 $mgkg^{-1}$ (2-15 $mgdm^{-2}$) were observed in the various food types studied which represents an 8-60% loss of DEHA from the film. Once again the results reflect the influence of temperature, exposure time and the form and content of fat in the food on the level of migration.

Table 18. Summary of Average Migration Values for DEHA and Reoplex 346 From Various Cling films into a Variety of Food.

Food type	Exposure		MIGRATION DEHA mgkg^{-1}	DEHA mgdm^{-2}	REOPLEX 346 mgkg^{-1}	REOPLEX 346 mgdm^{-2}
Cheese Slice	5 Days at 5°C	a)	246	5·1		
		b)			12·3	0·2
		c)	257	5·3	6·6	0·2
Egg Mayonnaise Sandwich	1 Day at 5°C	a)	41	1·9		
		b)			2·6	0·1
		c)	19	0·9	0·9	0·0
Chicken Breast (Complete dish)	Cover - microwave	a)	46	6·4		
		b)			8·4	1·3
		c)	18	2·5	4·3	0·6
Peanut biscuit	Lining of tin microwave	a)	362	15·0		
		b)			37·1	1·4
		c)	199	9·6	16·8	0·7
Swiss roll Whole	5 Days at 5°C	a)	37	2·0		
		b)			12·7	0·7
		c)	18	1·0	4·6	0·3
Swiss roll Slice	5 Days at 5°C	a)	226	7·1		
		b)			54·1	1·9
		c)	94	3·1	15·5	0·5

a - Conventional cling film (DEHA 17% w/w)
b - Polymeric plasticiser film (Reoplex 346 23 % w/w)
c - Mixed plasticiser film (DEHA 10%, Reoplex 346 10% w/w)
Results are averages of triplicate migration experiments

Table 19. Migration of DEHA From Conventional Cling film into a Variety of Food.

Food Type	Total Weight (g)	Exposure Details	Contact Area (dm^2)	MIGRATION of DEHA $mgkg^{-1}$	$mgdm^{-2}$
Cheddar Cheese	34	5 Days at 5°C	1·6	275	5·9
	33	in a sandwich type	1·6	215	4·4
	33	cell	1·6	248	5·1
Egg Mayonnaise Sandwiches	142	1 Day at 5°C	2·9	42	2·0
	137	Complete wrapping	2·9	44	2·1
	132	of the sandwich with overwrapping	2·9	37	1·7
Chicken Breast	149 *	Covered and cooked	1·5	40	3·9
	37 **	in a microwave oven		82	2·0
	168 *		1·5	39	4·3
	41 **			56	1·5
	206 *		1·5	† 36	4·8
	*			† 36	4·8
	*			† 35	4·7
	32 **			142	3·0
Peanuts Biscuits	118	Dish lined with	3·0	381	14·9
	134	film and cooked	3·0	362	16·0
	125	in a microwave oven	3·0	345	14·3
Swiss Roll	165	5 Days at 5°C	3·1	36	1·9
	162	Complete Swiss Roll	2·9	38	2·1
	162	wrapped with minimal overwrapping	2·9	37	2·1
	23	2cm Slice completely	0·8	233	6·5
	25	wrapped with minimal	0·7	220	7·8
	24	overwrapping	0·7	226	7·7

* meat
** cooking juices
† Analysis of replicate sub-sample

6.3.2.2 Polymeric Plasticised Film

The level of migration of R346 was considerably lower than that of DEHA under identical exposure conditions (table 20). Values were between 3 and 20 times lower than those for DEHA, at 1-54 $mgkg^{-1}$ (0·1-1·9 $mgdm^{-2}$) which represents between 0·3-5·7% depletion of R346 from the film. This reduction in migration may be due to the increased molecular size of R346 compared to DEHA, molecular weights are 1950 and 370 respectively, and is discussed in greater depth in the next chapter.

6.3.2.3 Mixed Plasticiser Film

The extent of migration of DEHA and R346 from the mixed plasticiser film is shown in table 21. The migration of each plasticiser was approximately one half of that seen from the films plasticised with DEHA or R346 alone. Since the individual levels of DEHA and R346 in the mixed plasticiser film were also approximately one half of that in the single plasticiser films, it would appear that the presence of one plasticiser does not markedly affect the migration of the other. This feature can be appreciated better from Figure 34, where the relative migration levels of DEHA and R346 are plotted, for the single plasticiser films versus the mixed plasticiser film. There is a direct and linear correlation between the probability of DEHA migration relative to R346 from the single plasticiser films and their migration from the mixed plasticiser film - again demonstrating that the migration of each plasticiser from the mixed film occurs independently of the other.

Table 20. Migration of Polymeric Plasticiser (Reoplex 346) From Cling Film into a Variety of Food.

Food Type	Total Weight (g)	Exposure Details	Contact Area (dm^2)	MIGRATION of Reoplex 346 $mgkg^{-1}$	$mgdm^{-2}$
Cheddar Cheese	33	5 Days at 5°C in a sandwich type cell	1·6	10·3	0·2
	34		1·6	11·0	0·2
	33		1·6	15·7	0·3
Egg Mayonnaise Sandwiches	142	1 Day at 5°C Complete wrapping of the sandwich with overwrapping	2·9	3·4	0·2
	130		2·9	2·0	0·1
	146		2·9	2·4	0·1
Chicken Breast	247 *	Covered and cooked in a microwave oven	1·5	8·4	1·3
	45 **			7·7	0·2
	156 *		1·5	10·5	1·1
	36 **			7·4	0·2
	210 *		1·5	7·3	1·0
	33 **			4·2	0·1
Peanut Biscuits	120	Dish lined with film and cooked in a microwave oven	3·0	37·4	1·5
	112		3·0	35·7	1·3
	105		3·0	38·3	1·3
Swiss Roll	160	5 Days at 5°C Complete Swiss Roll wrapped with minimal overwrapping	2·9	10·9	0·6
	166		3·1	11·3	0·6
	164		2·9	16·0	0·9
	22	2cm Slice completely wrapped with minimal overwrapping	0·7	47·8	1·6
	25		0·8	28·1	0·9
	26		0·7	86·4	3·2

* meat

** cooking juices

To ensure that the values obtained for DEHA contamination from mixed plasticiser film were correct, samples which had been exposed to a DEHA plasticised cling film were analysed using the polymeric plasticiser method. The results obtained were within ±4% of those obtained using the analytical method for DEHA analysis given in section 2.2.4. This is quite acceptable as the combined RSD of each method is 5%.

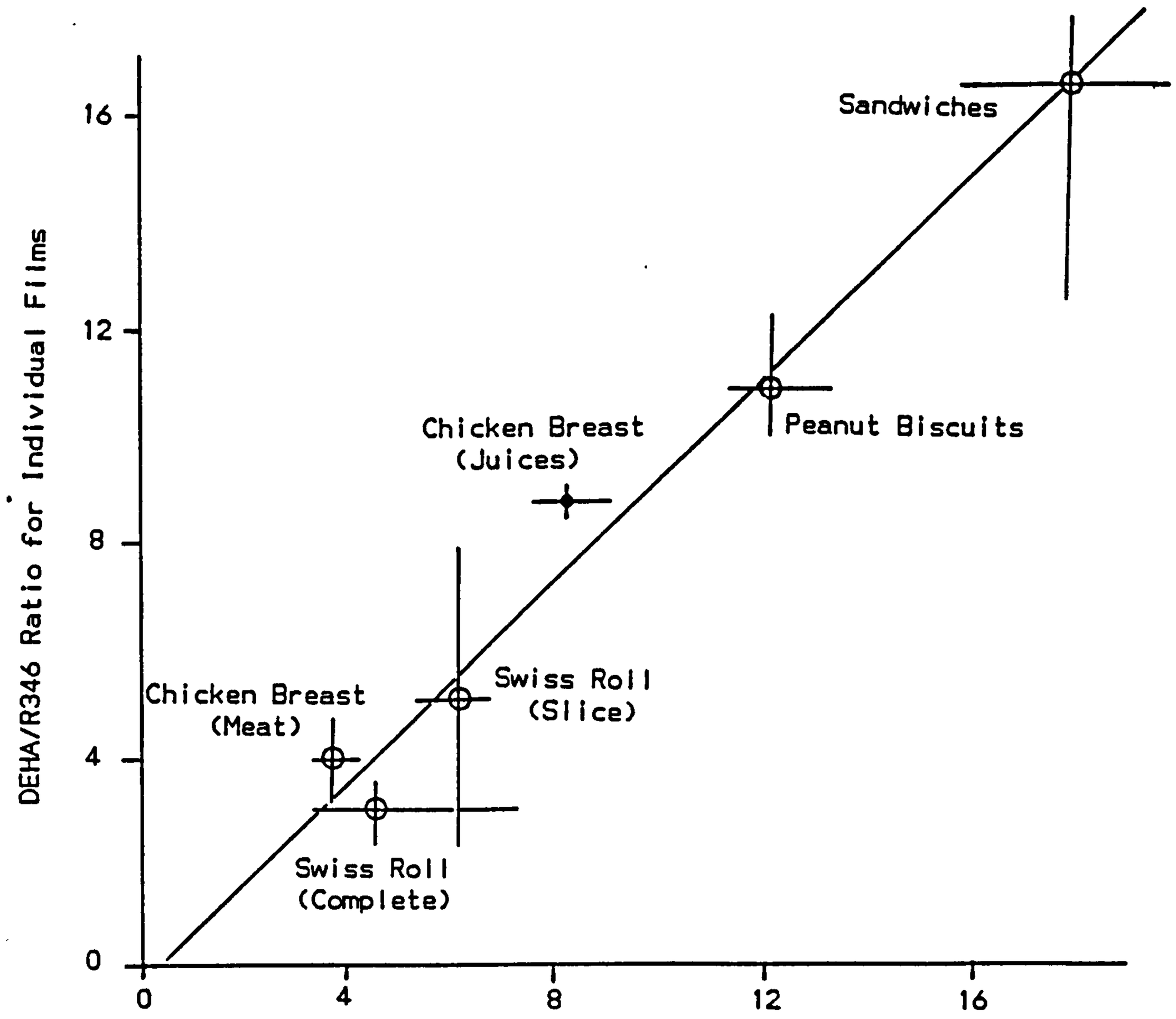
In summary the method of analysis for R346 in food, given in chapter five, was successfully used to determine the level of migration from R346 plasticised and R346 plus DEHA plasticised cling film into a variety of foods. In all cases the level of R346 migration into food, under identical exposure conditions, was less than that of DEHA, the levels being between three and twenty times lower.

Table 21. Migration of Polymeric Plasticiser (Reoplex 346) and DEHA From Mixed Plasticiser Cling film into a Variety of Food.

Food Type	Total Weight (g)	Exposure Details	Contact Area (dm^2)	MIGRATION DEHA $mgkg^{-1}$	DEHA $mgdm^{-2}$	REOPLEX 346 $mgkg^{-1}$	REOPLEX 346 $mgdm^{-2}$
Cheddar Cheese	33	5 Days at 5°C in a sandwich type cell	1·6	265	5·5	3·2	0·1
	34		1·6	222	4·7	9·6	0·2
	33		1·6	283	5·8	7·1	0·2
Egg Mayonnaise Sandwiches	139	1 Day at 5°C with Complete wrapping the sandwich with overwrapping	2·9	18	0·9	0·9	0·0
	122		2·9	16	0·7	0·4	0·0
	144		2·9	22	1·1	1·4	0·1
Chicken Breast	161 *	Covered and cooked in a microwave oven	1·5	17	1·8	4·8	0·5
	40 **			16	0·4	2·1	0·1
	223 *		1·5	13	1·8	3·8	0·6
	40 **			18	0·5	2·2	0·1
	158 *		1·5	22	2·3	5·2	0·5
	31 **			35	0·7	4·0	0·1
Peanut Biscuits	118	Dish lined with film and cooked in a microwave oven	3·0	200	7·9	17·6	0·7
	153		3·0	† 175	8·9	15·4	0·5
				† 213	10·8	16·1	0·8
				† 209	10·6	17·9	0·9
Swiss Roll	166	5 Days at 5°C Complete Swiss Roll wrapped with minimal overwrapping	3·1	17	0·9	5·0	0·3
	167		2·9	17	1·0	2·4	0·1
	162		2·9	20	1·1	6·5	0·4
	24	2cm Slice completely wrapped with minimal overwrapping	0·8	93	2·7	17·7	0·5
	21		0·7	87	3·1	13·2	0·5
	25		0·7	103	3·5	15·5	0·5

* meat
** cooking juices
† Analysis of replicate sub-samples.

Figure 34. Correlation of the Ratio of DEHA and R346 Migration from Single and Mixed Plasticiser Cling Films.



Y-axis : Ratio of the migration of DEHA ($mgdm^{-2}$) from conventional film to that of R346 from the polymeric plasticiser film. Migration levels determined in parallel but separate experiments.

X-axis : Ratio of the co-migration levels of DEHA and R346 from the mixed plasticiser film.

The centre of each "cross" represents the x-y coordinates of the mean for each food type. The associated "bars" represent the range of values seen for each food type (triplicate experiments).

Slope	= 0·945
Correlation coefficient	= 0·9876
Intercept (x=0)	= -0·25

CHAPTER 7

DETERMINATION OF THE MOLECULAR SIZE DISTRIBUTION OF THE POLYMERIC PLASTICISER REOPLEX 346 WHICH MIGRATES FROM PVC CLING FILM INTO FOOD

7.1 INTRODUCTION

The level of migration of the polymeric plasticiser R346 from PVC cling film into food was found to be up to twenty times less than that of DEHA under identical exposure conditions. This reduced level of migration was due to the size greater molecular size of R346 compared to DEHA. A number of studies have shown that the level of phthalate plasticiser migration decreases with increasing molecular weight (136, 137).

R346 is an uncapped condensation polyester and thus consists of a range of oligomers with various chain lengths. If molecular size is a controlling factor in migration of individual R346 oligomers, and polymeric plasticisers in general, then the relative migration of the small oligomers would be greater than that of the large oligomers.

Preferential migration of the small R346 oligomers could have implications for the assessment of the risk associated with dietary intake of polymer plasticisers. Thus the relationship between molecular size and level of individual R346 oligomer migration into food was investigated. It was also hoped that this information would enable the prediction of polymeric plasticiser migration into food.

The molecular size distribution of R346 which migrated into olive oil was studied using SEC. This technique separates compounds on the basis of size, the molecules eluting from the column in decreasing molecular size. All of the sample elutes in one column volume, large molecules eluting in the void or exclusion volume (V_o) and small molecules

eluting in the inclusion volume (V_i). More complete descriptions of SEC are available in the literature (138, 139).

SEC is practised in two forms; open column SEC (OCSEC) and a high performance variation that employs HPLC techniques, HPSEC. The essential difference between the two forms of SEC is the particle size of the gels. HPSEC utilizes smaller particles than OCSEC which produces columns of much greater efficiency, up to 70,000 theoretical plates per metre for 5 µm gels (140).

HPSEC was used to determine the molecular size distribution of R346 and OCSEC the determination of the molecular size distribution of R346, R346 extracted from R346-plasticised PVC cling film and R346 which had migrated into olive oil. The samples were fractionated with OCSEC, the fractions transmethylated and the mass of R346 in each fraction determined, as DMA, by GC-FID.

7.2 EXPERIMENTAL

7.2.1 MATERIALS

7.2.1.1 Primary Standards

The following primary standards were available commercially; DCHP (British Cellophane Ltd.), DEHP (Genomoll 100, Hoechst), DEP (Bisoflex, BP), DMPim (Aldrich Chemical Company), R346 (Ciba-Geigy), 1,2-dichlorobenezene and polystyrene Mwt. approximately 100,000 daltons (BDH Chemical Company). All standards, except R346 and

polystyrene which were not amenable to GC analysis, were better than 96% pure when analysed by capillary GC.

7.2.1.2 Internal Standard

Di(2-ethylhexyl) pimelate (DEHPim) was synthesised by the esterification of diethylpimelate (0·6g) with 2-ethyl-1-hexanol (4 mL) and BF_3 etherate (0·5 mL) in a capped vial at 100°C for 24 hours. The sample was diluted with hexane (4 mL), washed with distilled water (4 x 2 mL) and dried (Na_2SO_4). The hexane layer was then decanted and evaporated to dryness under nitrogen.

7.2.1.3 Reagents and Solvents

The following reagents were commercially available; BF_3 etherate (Sigma Chemical Company), BF_3-MeOH complex ca. 12% w/w BF_3 and 2-ethyl-1-hexanol (Aldrich Chemical Company). All solvents were of HPLC-grade and supplied by Rathburn (Walkerburn, Scotland) unless stated otherwise.

7.2.2 <u>INSTRUMENTATION</u>

High Performance Size Exclusion Chromatography

Columns	: 2µm pre-column filter in series with a PL Gel 500Å x 30 cm in series with 2 x PL Gel 100Å x 30 cm (Polymer Laboratories)
Pump	: SP8 700XR extended range LC pump with an SP8 500 dynamic mixer (Spectra Physics)
Injection system	: 7125 (Rheodyne) with a 10µL loop
Detector	: Differential Refractometer R401 (Waters)
Chart recorder	: BD41 (Kipp & Zonen)

7.2.3 DETERMINATION OF MOLECULAR SIZE DISTRIBUTION

7.2.3.1 R346 by HPSEC

R346 (0·5 mg) was loaded onto the HPSEC system described in section 7.2.2 and eluted with tetrahydrofuran (1·5 mLmin^{-1}). Polystyrene (Mwt 100,000 daltons) and 1,2-dichlorobenezene were used to determine V_o and V_i, respectively, of the system.

7.2.3.2 R346 by OCSEC

A sample of R346 (65 mg), DCHP (0·20 mg), DEP (0·16 mg) and DEHP (0·16 mg), as UV-active retention markers, was loaded onto the automated SEC system outlined in section 2.2.2.1 and the effluent monitored at 254nm. Two-minute fractions (6 mL) of the effluent were collected between 24-102 minutes and DMPim (6·6 mg) added to each as an internal standard. The samples were vortex-mixed to ensure homogeneity of the analyte and internal standard and then quantitatively transferred to a vial with acetone and blown to dryness under nitrogen. The samples were then transmethylated and extracted as in section 5.2.2 and analysed directly by GC using the system described in section 3.2.2.1 with the column held isothermally at 110°C.

7.2.3.3 R346 Extracted from R346-Plasticised PVC Cling Film

A sample of R346-plasticised PVC cling film (1g) was extracted by shaking with chloroform (15 mL) for 24 hours. The film was then removed from the chloroform and the sample evaporated to dryness under nitrogen. The residue was redissolved in DCM/C_6H_{12} (6·7 mL, 1:1 v/v), centrifuged and the supernatant passed through a 2µm filter (ACRO LC13 Gelman Sciences). Multiple injections (1 mL) of the supernatant onto

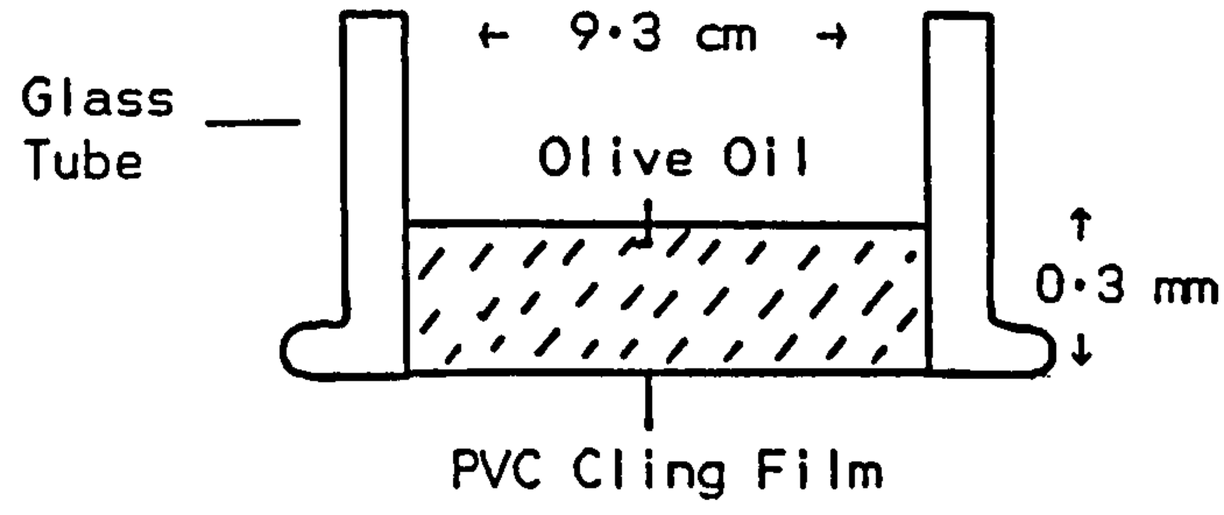
the automated OCSEC system were then made and 3 mL fractions of the eluant were repetitively collected between 25-86 minutes.

DEHPim (3·6 mg) was added to each fraction, as an internal standard, and the fractions were vortex-mixed, evaporated to a small volume under nitrogen, transferred to a vial (1·5 mL) with DCM/C_6H_{12} (1:1 v/v), and then evaporated carefully to dryness under nitrogen. The samples were then transmethylated with BF_3-MeOH (14% BF_3 v/v, 400 µL) for two hours at 60°C, quenched with distilled water (300 µL) and extracted with DCM/C_6H_{12} (900 µL, 7/3 v/v). The sample was centrifuged to aid phase separation and the organic phase was analysed by GC using the system described in section 3.2.2.1 with a fused silica bonded phase CP-Sil 5 CB 50m x 0·32mm 1·2µm film thickness column (Chrompack) and the following column temperature programme: 160°C for 10 min, 30°Cmin^{-1} to 280°C and hold 5 min.

7.7.2.4 R346 Migration from Cling Film into Olive Oil

R346-plasticised PVC film (33 mgdm^{-2} R346) was exposed to a thin film of olive oil ('Lanfranchi' Leon Frenkel Ltd. Kent), as illustrated in figure 35.

Figure 35. Cross-section (view) of the Experimental Arrangement for the Exposure of PVC Cling Film to Oil.



Exposure was at 23°C ± 0·1 for twelve hours after which the oil was removed from the surface of the film by syringe. Approximately 80% of the oil was recovered. A small aliquot (50 µL) of oil was then analysed for total R346 using the method described in section 5.2.2. The remaining oil was diluted to 0·5 mgL^{-1} with DCM/C_6H_{12} (1:1, v/v) and an aliquot (1 mL) fractionated using the automated OCSEC system (section 2.2.2.1) into 3 mL fractions collected between 25-85 minutes. Fractions for five repeat injections were pooled.

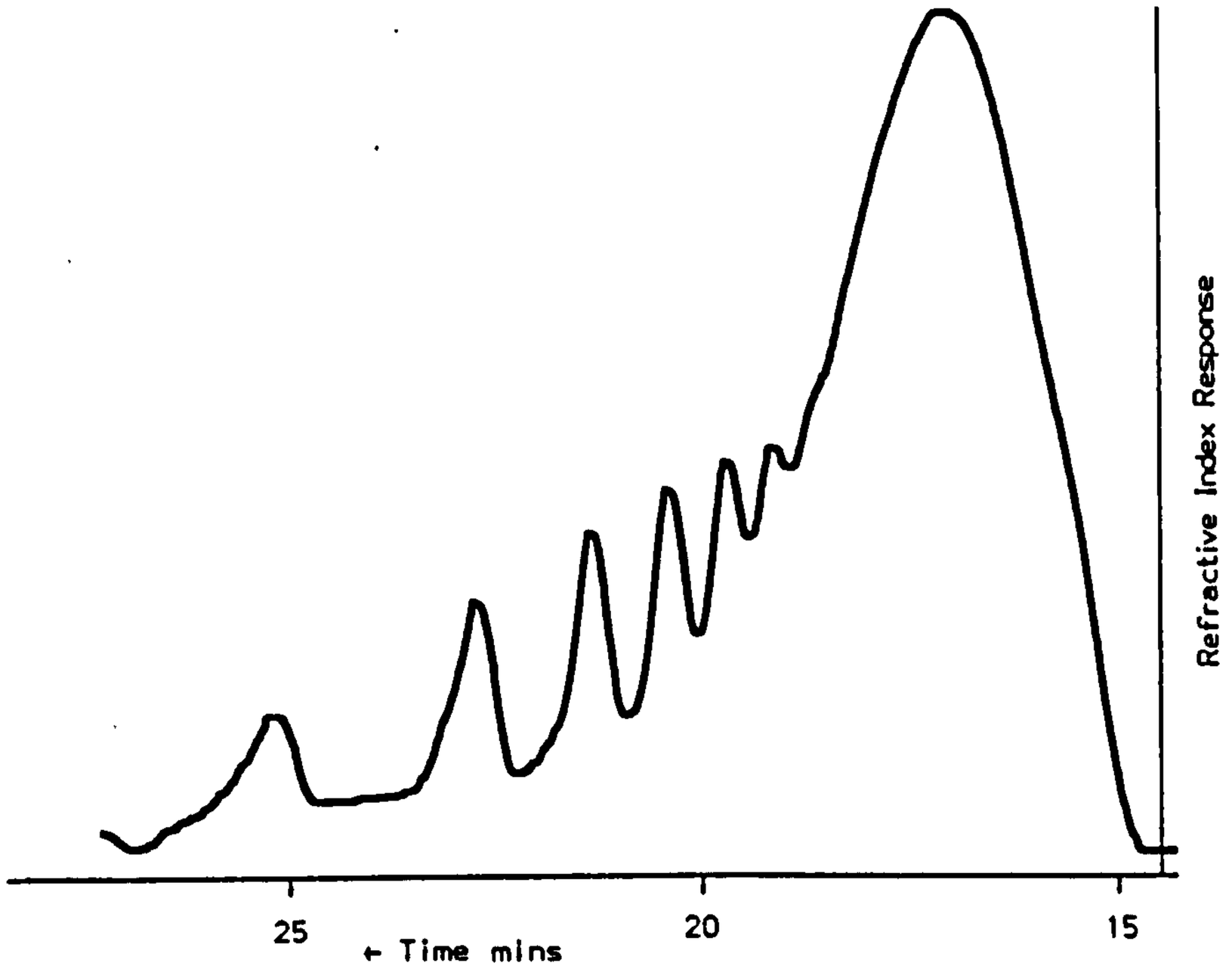
7.3 RESULTS AND DISCUSSION

7.3.1 DETERMINATION OF THE MOLECULAR SIZE DISTRIBUTION OF R346 BY HPSEC/REFRACTIVE INDEX DETECTION

R346 contains a series of at least six oligomers of different molecular size (figure 36). Approximately 75% of the sample eluted close to the exclusion volume of the system and was too large to be effectively separated on the HPSEC columns available. However, the remaining 25% of the sample was resolved into five peaks. The molecular size distribution observed for R346 was identical to that of a polymeric plasticiser commercially used in PVC cling film (figure 37) indicating that this study of R346 is of direct relevance to plastic packaging used currently.

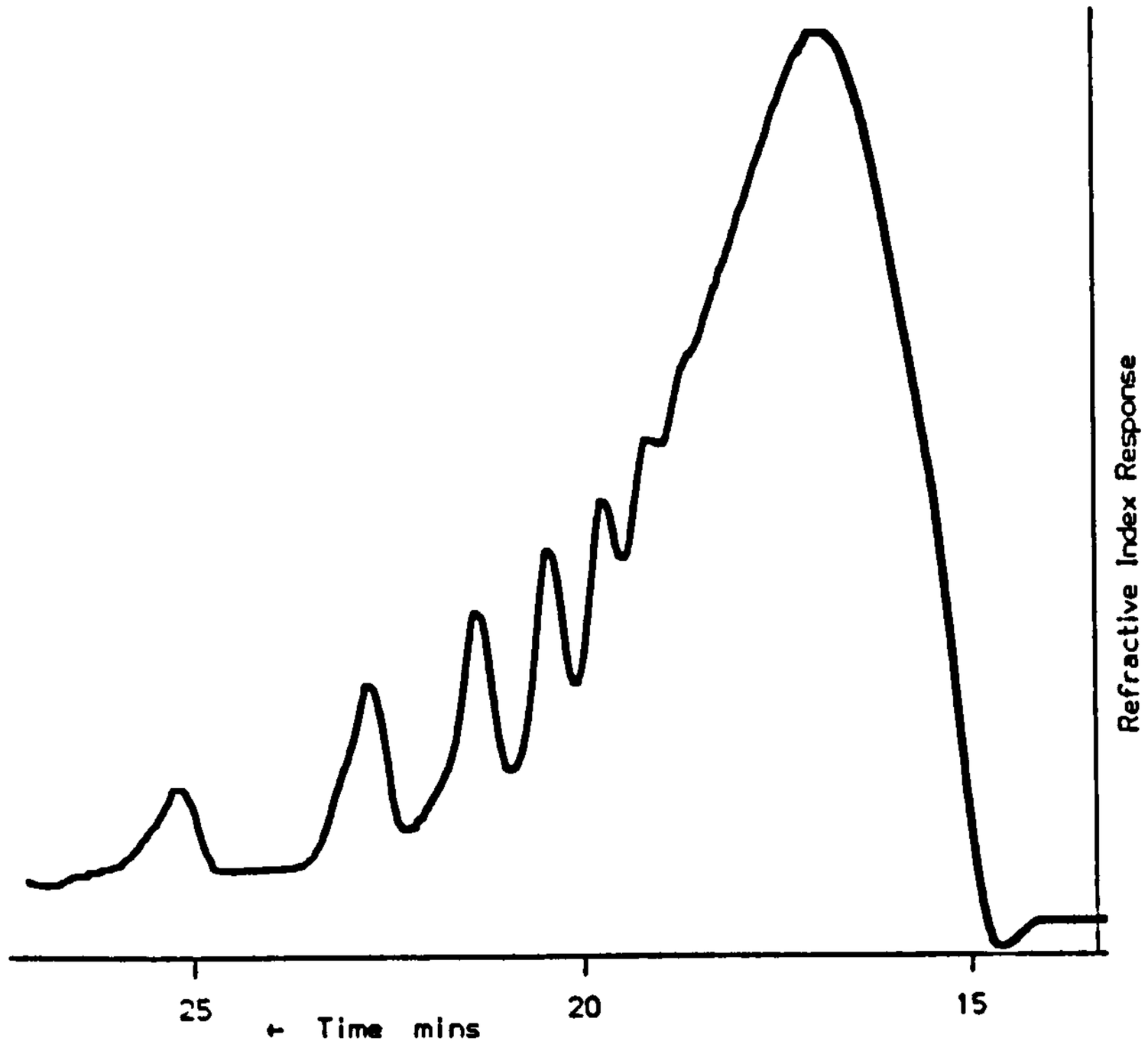
Although HPSEC was successfully employed in the determination of the molecular size distribution of a standard of R346, it was unsuitable for the analysis of R346 in food because of the limited sample

Figure 36. HPSEC Chromatogram of R346.



Chromatographic conditions: PL Gel 500Å x 30 cm in series with 2 x PL Gel 100Å x 30 cm columns operated at 1 mLmin^{-1} DCM/C_6H_{12} (1:1, v/v) with refractive index detection.

Figure 37. HPSEC Chromatogram of a Polymeric Plasticiser Commercially used in PVC Cling Film.



Chromatographic conditions: PL Gel 500Å x 30 cm in series with 2 x PL Gel 100Å x 30 cm columns operated at 1 mLmin^{-1} DCM/C_6H_{12} (1:1, v/v) with refractive index detection.

capacity of HPSEC columns. OCSEC, which has a greater sample loading capacity (up to 500 mg (141)), was used for the further work.

7.3.2 DETERMINATION OF THE MOLECULAR SIZE DISTRIBUTION OF R346 BY OCSEC WITH GC-FID DETECTION

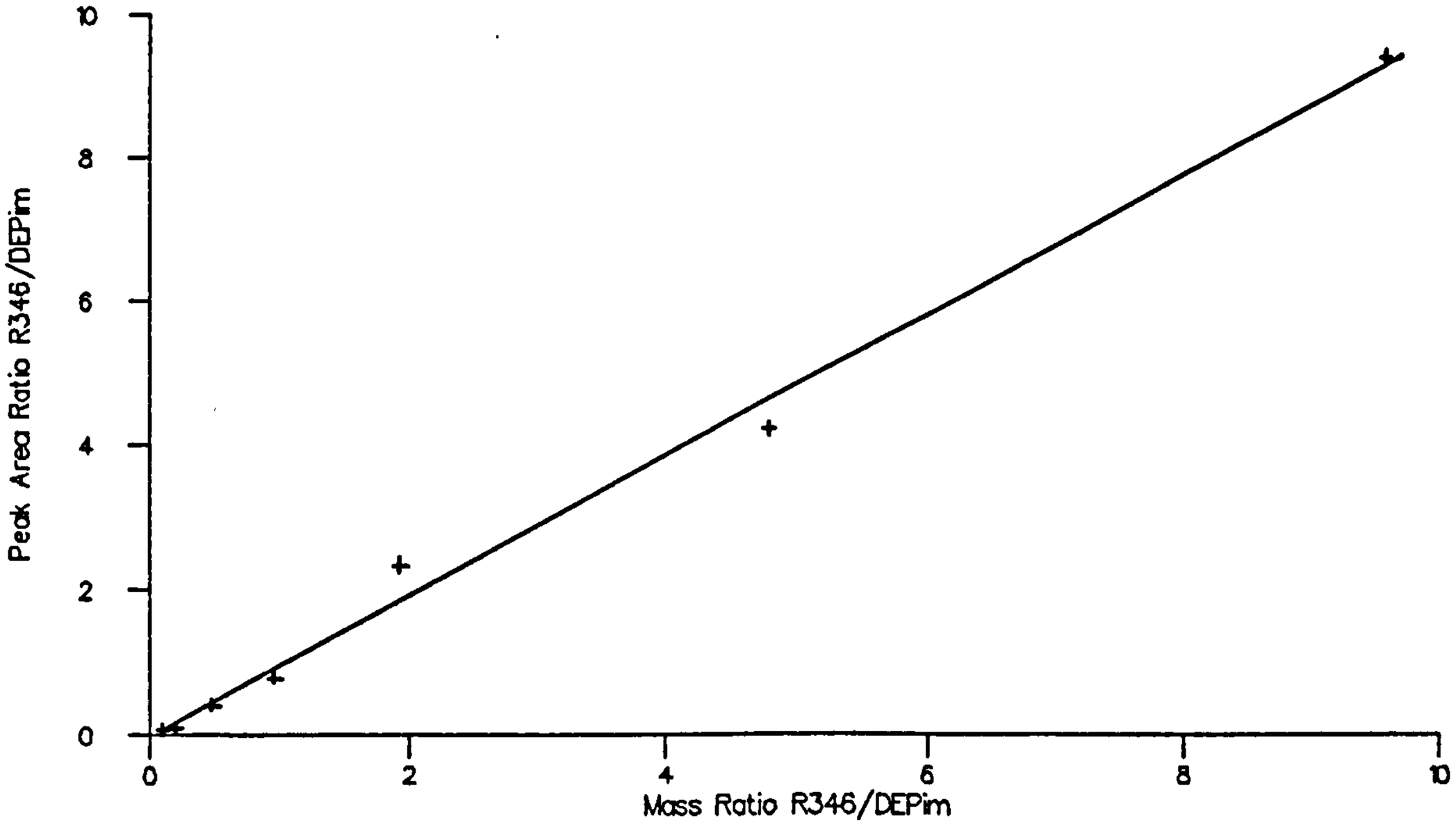
7.3.2.1 Method

As refractive index is a non-specific detection technique and unable to distinguish R346 oligomers from food or other film components an alternative method of detection was required. The method of analysis given in chapter 5 is suitable, but the use of GC-MS end determination means that the analysis of a large number of samples would be costly and labour intensive as no automated GC-MS facilities were available. Thus, the method was modified to take advantage of automated GC-FID facilities. The final method involved the separation of R346 oligomers by OCSEC, transmethylation of each fraction and then the analysis for R346, as DMA, by GC-FID.

Choice of Internal Standard

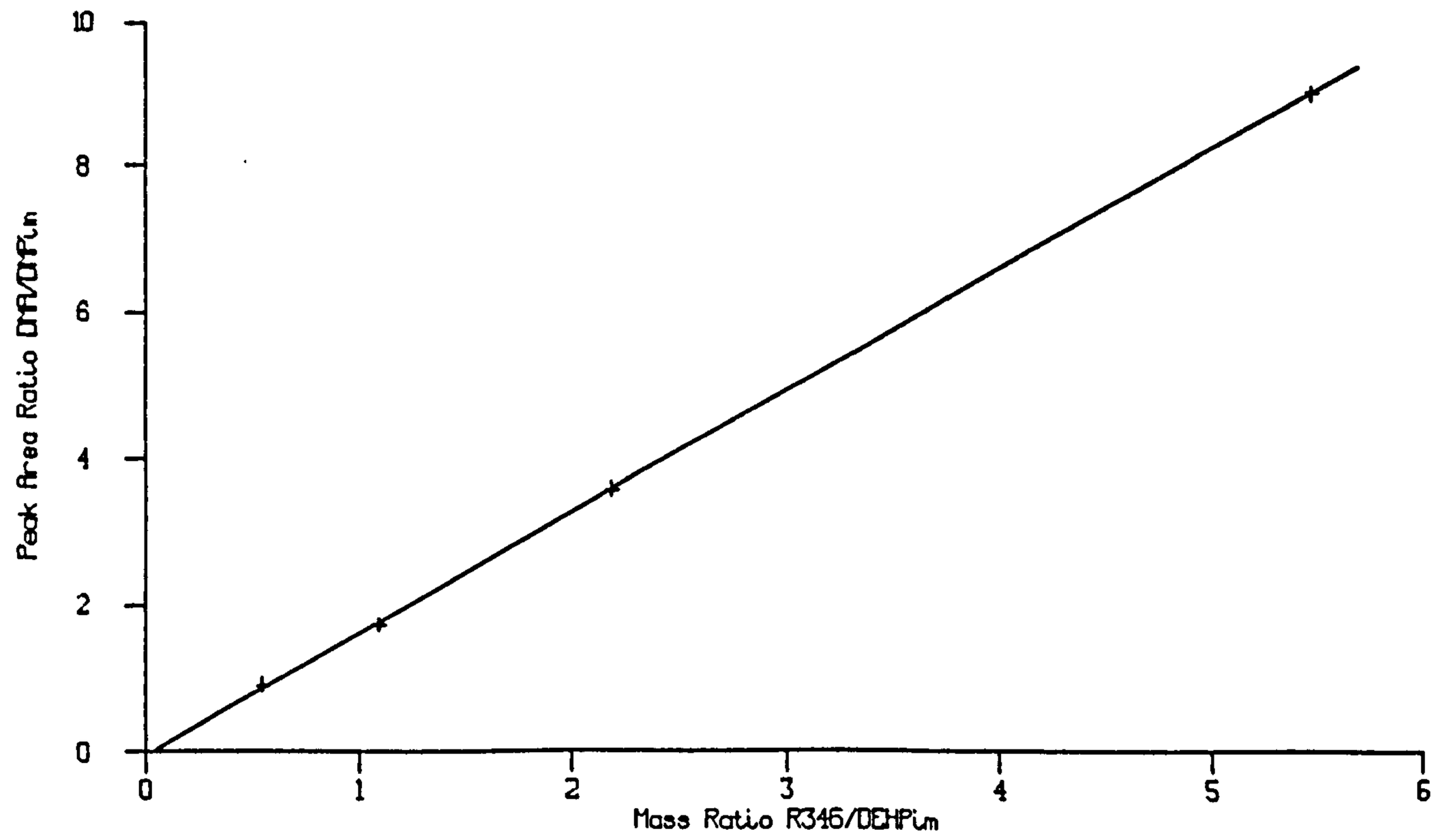
Initially DMPim was used as the internal standard. A straight line calibration graph (slope = 0·972 and a correlation coefficient of 0·9969) was obtained for mixtures of R346 and DMPim in known ratios (figure 38) and DMPim was successfully used in the analysis of a R346 standard. However, it was felt that an internal standard which underwent transmethylation, thereby allowing for any losses that might occur at that stage, would be preferred. DEHPim was chosen as an alternative internal standard as it was analogous to d_4-DEHA which had been successfully used in analysis of R346 in food (chapter 6) and would undergo transmethylation. The calibration curve obtained for

Figure 38. Calibration Graph for the Analysis of R346, as DMA, by GC using DEPim as an Internal Standard.



Slope = 0.972
Correlation Coefficient = 0.9969
Intercept (x = 0) = −0.02

Figure 39. Calibration Graph for the Analysis of R346, as DMA, by GC with DEHPim as an Internal Standard.



Slope = 1.66
Correlation Coefficient = 0.9999
Intercept (x = 0) = −0.06

mixtures of DEHPim and R346 in known ratios was a straight line with a slope of 1.66 and a correlation coefficient of 0·99996 (figure 39).

The retention time of the analyte and internal standard peaks of the samples were within 2% of those of the standards. No interfering peaks were observed in the GC chromatogram and, as can be seen from figure 40, the peaks were well resolved.

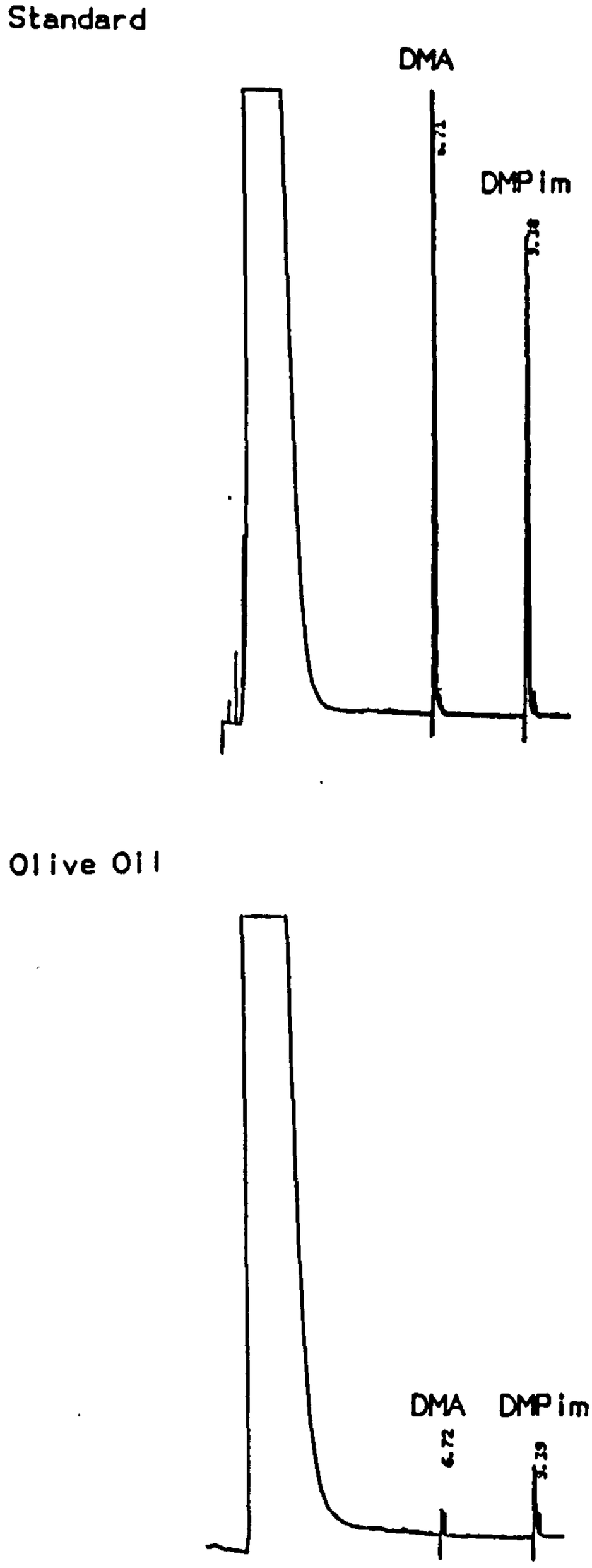
7.3.2.2 SEC Profile of R346 Standard

The majority (80%) of the sample eluted in a single peak close to the molecular weight exclusion limit of the column which is approximately 2,000 daltons. This is consistent with the weight-average molecular weight ($\bar{M}_w$) and the number-average molecular weight ($\bar{M}_n$) of R346 which is 4,060 and 1,950 daltons respectively. In addition to this excluded peak there was a series of five smaller peaks attributed to small oligomers (figure 41). These results from the OCSEC compare well with HPSEC results (figure 36).

7.3.2.3 SEC Profile of R346 in PVC Cling Film

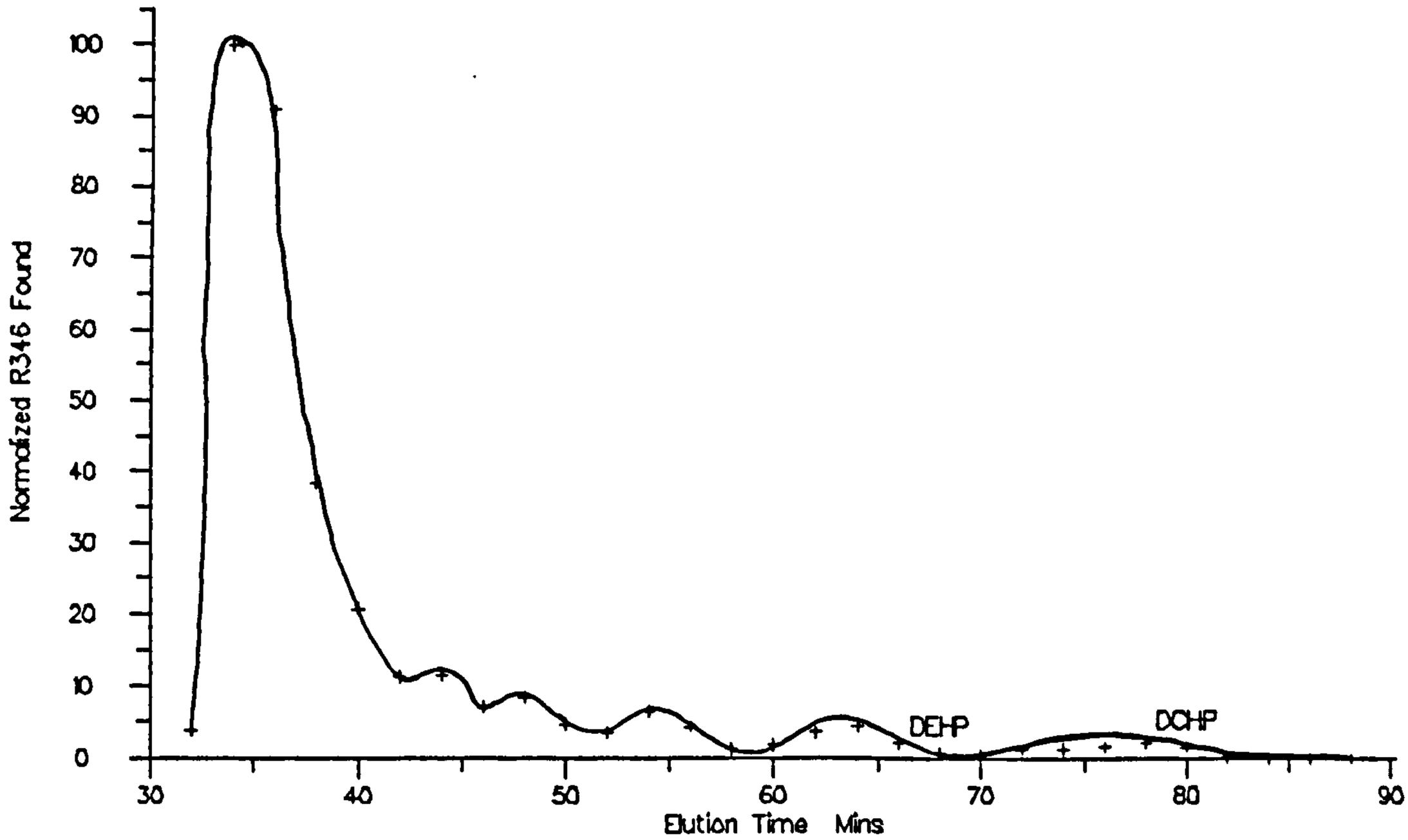
The SEC profile of R346 extracted from food grade PVC cling film (figure 42) was less regular than that for the R346 standard and this is undoubtedly due to the presence of coextracted PVC oligomers and low molecular weight additives such as epoxidised soya bean oil (ESBO). However, the major features of R346 were present with the pattern of one major peak at V_o followed by a series of five smaller peaks of decreasing peak area demonstrating there was no appreciable loss of R346 oligomers during film manufacture.

Figure 40. Gas Chromatographic Analysis of Olive Oil for R346, as DMA.



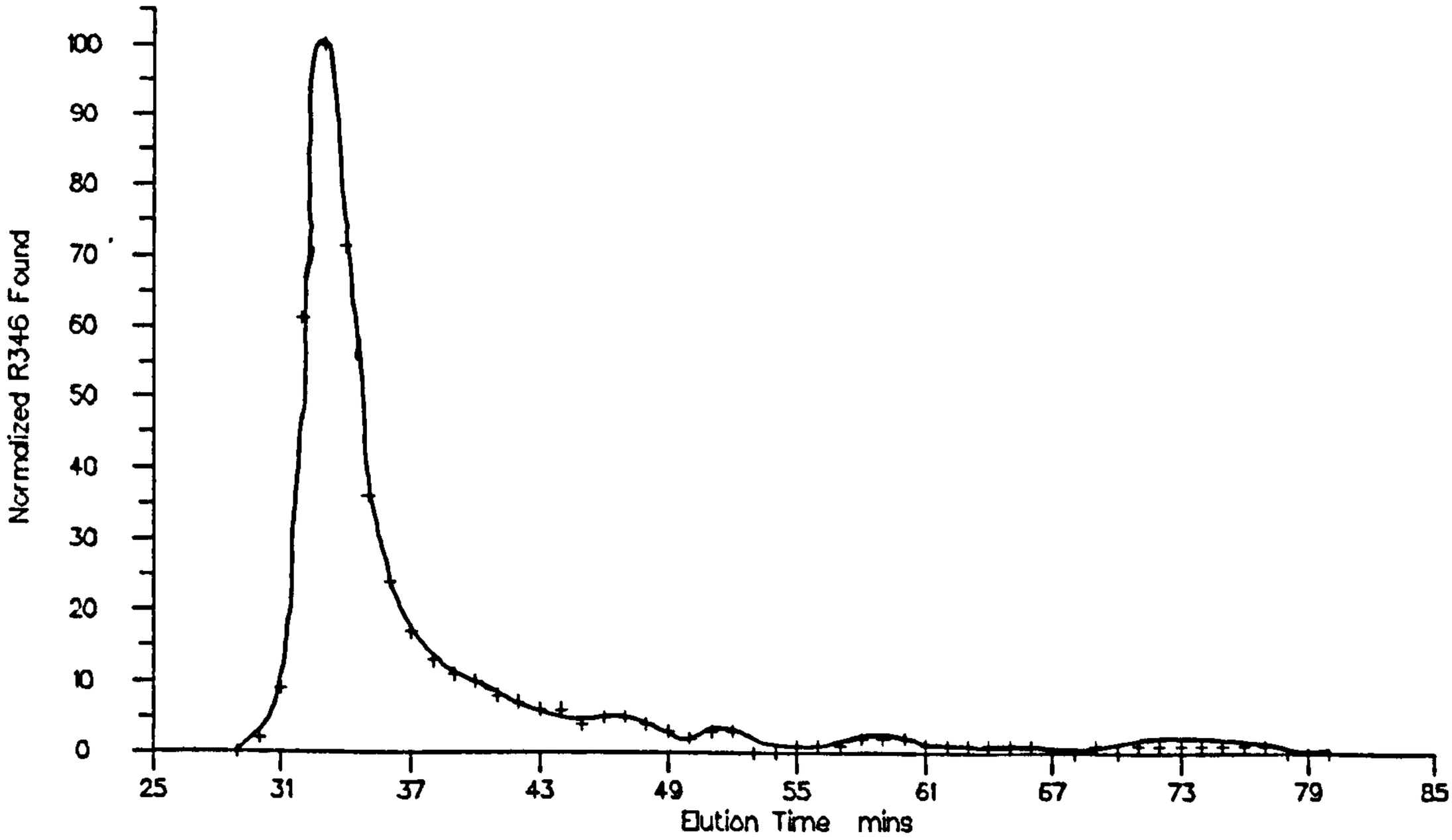
GC conditions: 25m × 0·23mm I.D. CP SIL 5CB fused silica column operated using hydrogen carrier gas at 3 mLmin^{-1} with FID. The column was held isothermally at 110°C.

Figure 41. The Molecular Size Distribution of R346 by OCSEC.



Chromatographic conditions: 1mx25mm I.D. column packed with 800 mm bed of SX–3 (Bio–Beads) eulted with dichloromethane/cycloheane (1:1, v/v) 3 mL.min–1

Figure 42. The Molecular Size Distribution of R346 Extracted from R346 Plasticised Cling Film by OCSEC.



Chromatographic conditions: 1mx25mm I.D. column packed with 800 mm bed of SX–3 (Bio–Beads) eulted with dichloromethane/cycloheane (1:1, v/v) 3 mL.min–1

7.3.2.4 SEC Profile of R346 after Migration into Olive Oil

The concentration of R346 in olive oil exposed to R346-plasticised PVC cling film for twelve hours at 23°C was 390 $mgkg^{-1}$. This represented a loss from the film of 1·07 $mgdm^{-2}$. The maximum loss of R346 from the film under simulated home use was 3·2 $mgdm^{-2}$ (table 18). The high concentration of R346 in the oil was achieved by exposing a thin layer of oil to the film producing in a high contact area to mass ratio. The molecular size distribution of the R346 oligomers which migrated from the film into the olive oil contained three broad peaks (figure 43).

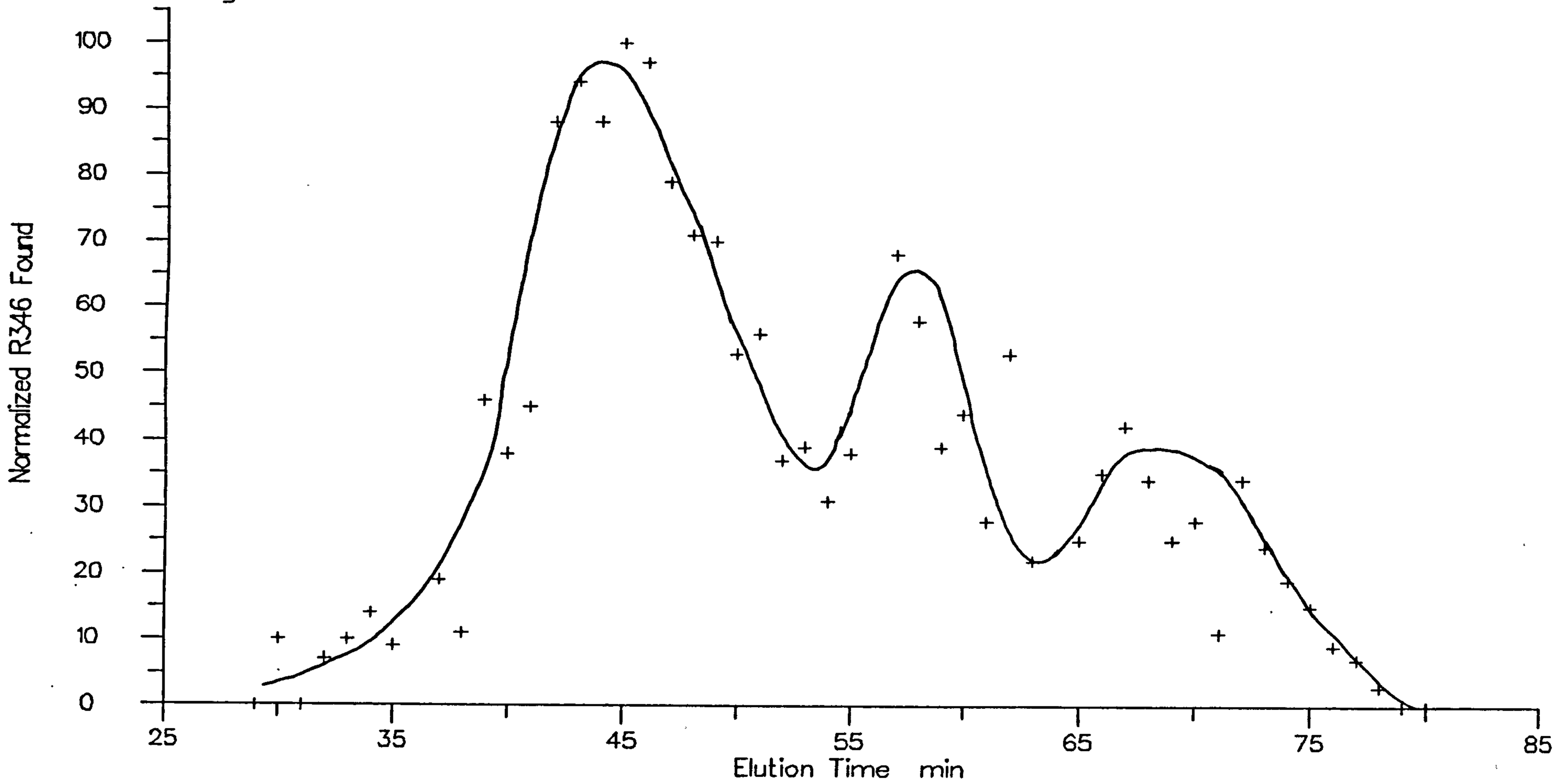
7.3.3 COMPARISON OF THE MOLECULAR SIZE DISTRIBUTION OF R346 AND R346 WHICH MIGRATES INTO OLIVE OIL

There was a marked difference in the molecular size distribution of the oligomers which migrated into olive oil compared to a R346 standard (figure 44). The difference can be more clearly seen when the mass of R346 oligomer, expressed as a percentage of the total mass found, in each of the major peaks is compared for the migrated and standard R346 (figure 45). There was no appreciable migration of the larger oligomers and the smaller oligomers, peaks 3 and 4, represented 34% of R346 found, compared to 6% for the standard.

The differences in the molecular size distribution of the migrated oligomers and standard is probably due to differences in the mobility of the various oligomers within the PVC.

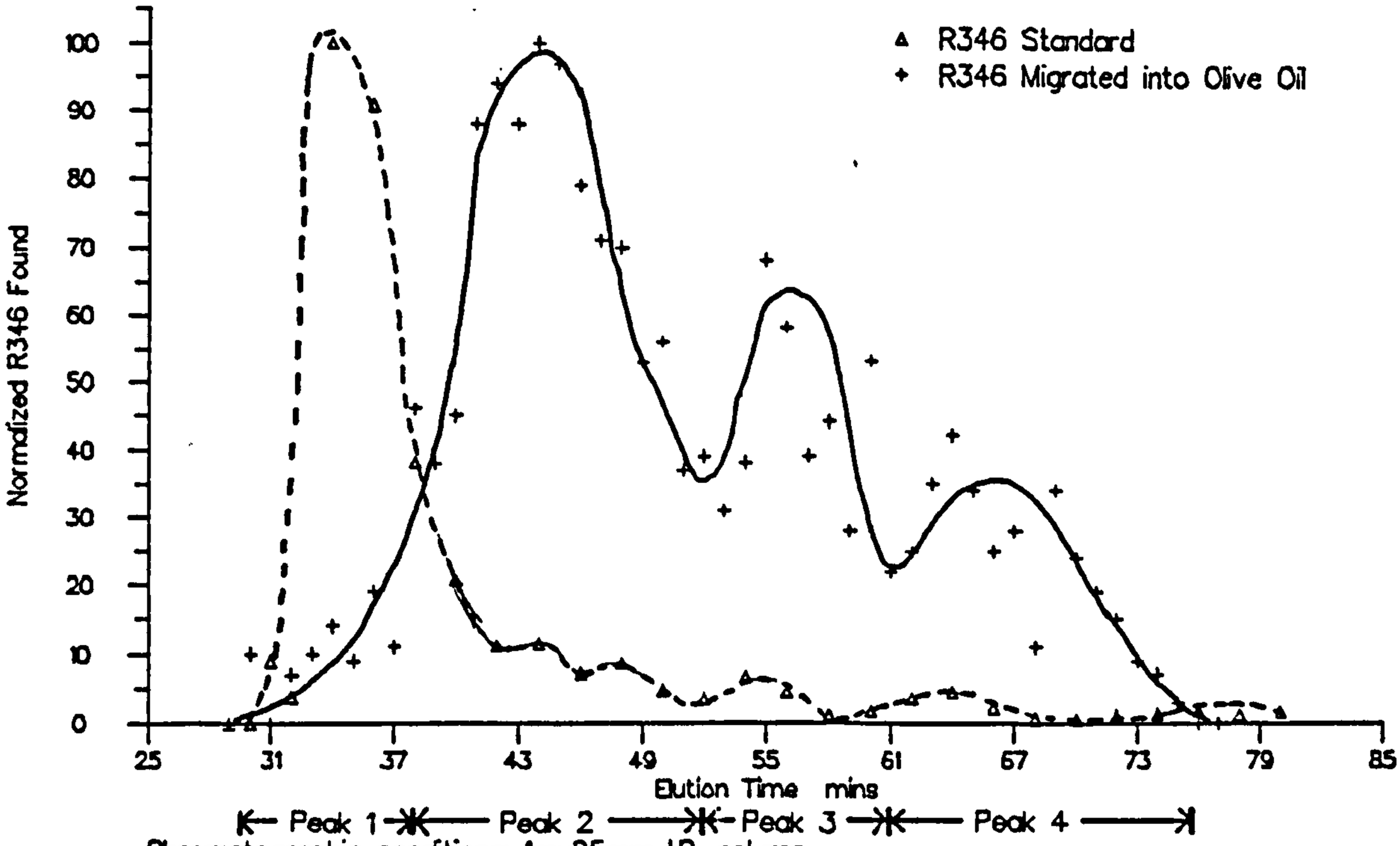
For a static system, that is one which is not agitated, additives are transported through the system by diffusion (142). There are three

Figure 43. The Molecular Size Distribution of R346 that Migrated from PVC Cling Film into Olive Oil.



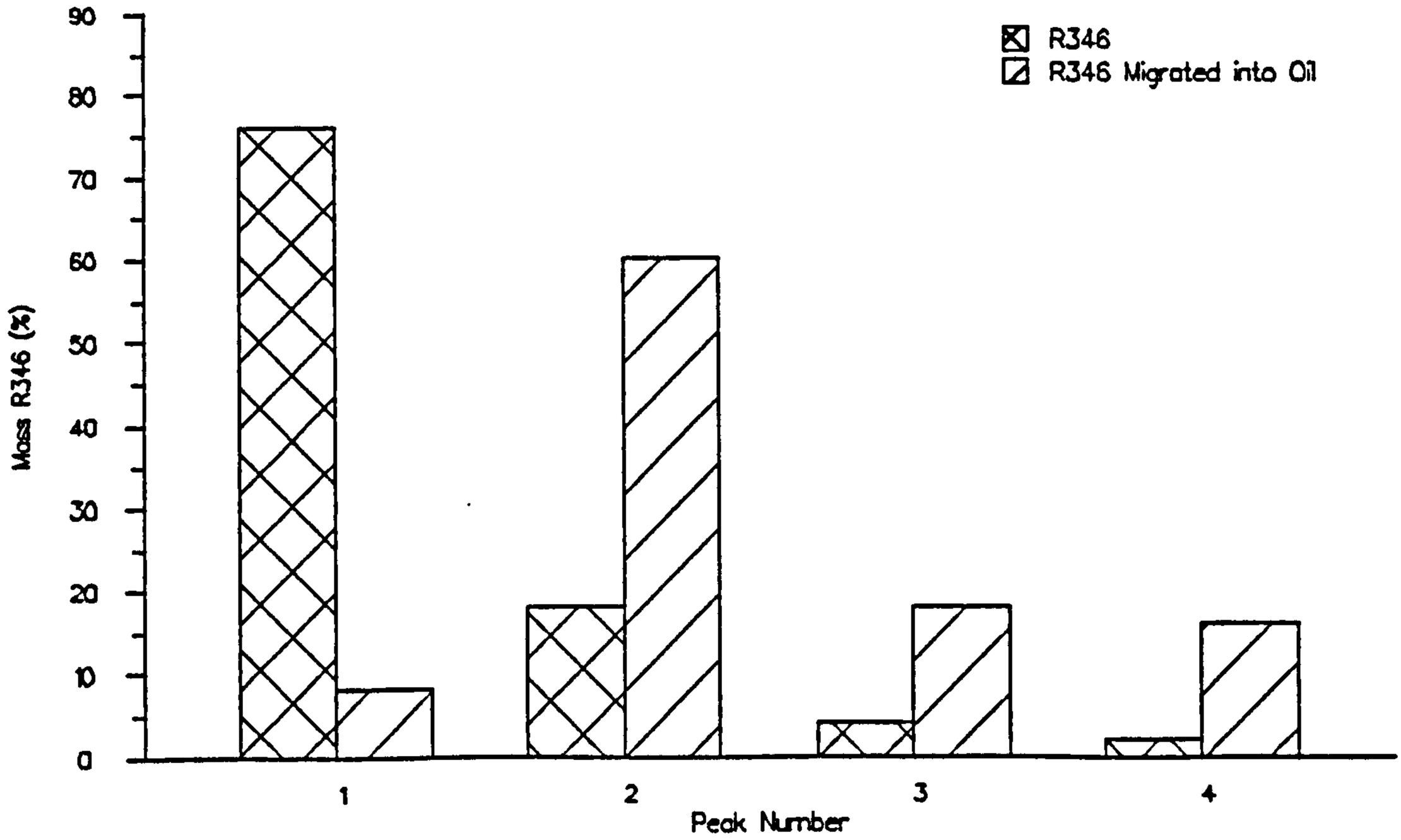
Chromatographic conditions: 1mx25mm I.D. column packed with 800 mm bed of SX–3 (Bio–Beads) eulted with dichloromethane/cycloheane (1:1, v/v) 3 mLmin–1

Figure 44. Comparison of the Molecular Size distribution of R346 and R346 that Migrated into Olive Oil.



Chromatographic conditions: 1mx25mm I.D. column packed with 800 mm bed of SX–3 (Bio–Beads) eulted with dichloromethane/cycloheane (t:1, v/v) 3 mLmin–1

Figure 45. Relative Mass of R346 which Elutes in Peaks One to Four for R346 and R346 which Migrated into Olive Oil.



Mass R346 (%) is the mass of R346 found in the given peak as a percentage of the total mass of R346 found.
The elution times of the peaks are given in figure 44.

possible mechanisms of diffusion of long-chain (polymeric) molecules through a polymer matrix; reptation, constraint release and Stokes-Einstein diffusion (143). The diffusion mechanism which occurs is dependant on the the molecular weight of the polymeric molecule (M) compared to that of the polymer matrix (P) and the critical molecular weight (M_c) required to produce an entangled network. When $P>M>M_c$ then diffusion is by reptation, when $M>P>M_c$ diffusion is by constraint release and when $M>M_c>P$ diffusion is by Stokes-Einstein diffusion (144).

The diffusion of R346 in PVC should be by reptation as the average molecular weight of PVC is 60,000 to 150,000 daltons (145) and $\bar{M}_w$ of R346 is 4,060 daltons. A molecule which diffuses by reptation can be considered as a number of connected segments which must move in a coordinated manner in order for the whole molecule to move. This type of motion has been likened to the movement of a snake. The relationship between the molecular weight (M) and the diffusion coefficient (D) of a molecule which diffuses by reptation is given by equation 13 (143).

$$D \propto M^{-2} \qquad (13)$$

If the ease of migration is directly proportional to D then the percentage of each oligomer which migrates (M_p) will be inversely proportional to the square of it's molecular weight (equation 14).

$$M_p \propto M^{-2} \qquad (14)$$

This hypothesis may be tested by considering the percentage migration of the oligomers in peaks one and four. Assuming that the average molecular weight of the oligomers in peak one is 4,000 daltons based on $\bar{M}_w$ = 4,060 daltons and $V_o \simeq$ 2,000 daltons, and the average

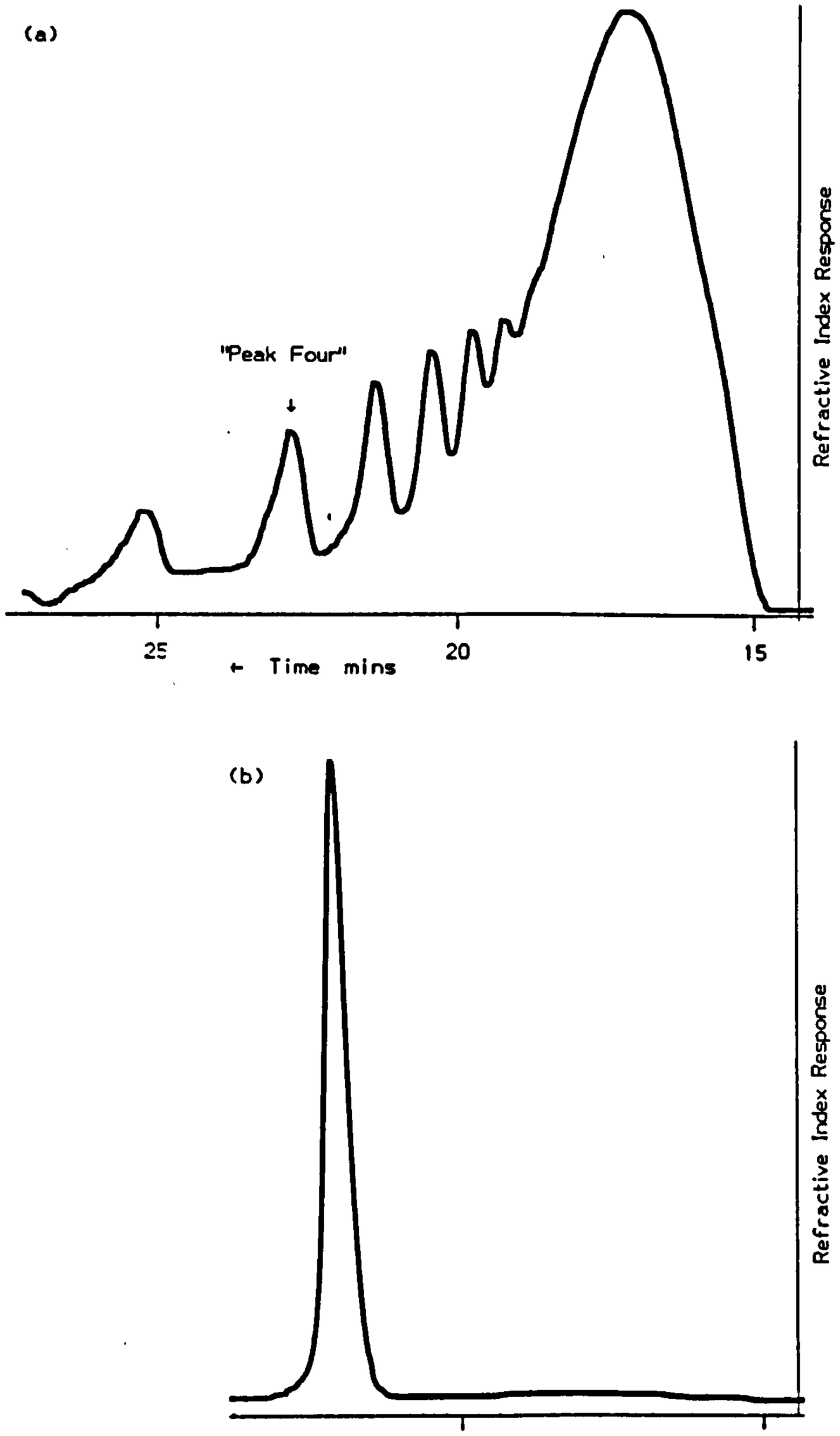
molecular weight of the oligomers in peak four is 350 based on the elution volumes of DEHA, DCHP and DEP (figures 46 and 41). Then equation 14 predicts that the percentage migration of the oligomers in peak four will be 130 times greater than that of the oligomers in peak one. The observed R346 migration into olive oil, section 7.3.2.4, shows excellent agreement with this, the percentage migration of the oligomers in peak four being 110 times that of the oligomers in peak one, table 22.

Table 22. Level of Individual R346 Oligomer Migration into Olive Oil.

Peak Number	% of Available Oligomer Which Migrated into Oil	Ratio Oligomers Migrated into Oil
1	0.1	= 1
2	6.1	44
3	8.4	60
4	15.3	110

Thus the migration of polymeric plasticisers from PVC cling film into food is a diffusion process and the percentage migration of individual oligomers is inversely proportional to the square of their molecular weight.

Figure 46. Estimation of the Molecular Size of the R346 Oligomers which elute in Peak Four.



(a) HPSEC chromatogram of R346.

(b) HPSEC chromatogram of DEHA.

Chromatographic conditions: PL Gel 500Å x 30 cm in series with 2 x PL Gel 100Å x 30 cm columns operated at 1 mLmin^{-1} DCM/C_6H_{12} (1:1, v/v) with refractive index detection.

7.3.4 PREDICTION OF R346 MIGRATION BASED ON DEHA MIGRATION FOR IDENTICAL EXPOSURE CONDITIONS OF FOOD TO FILM

As discussed in the previous section, the oligomers which elute in peak four are of a similar size to DEHA. Assuming that these oligomers migrate into food to the same extent as DEHA, under identical exposure conditions, and that the remaining oligomers migrate in the same ratio as into olive oil. R346 migration into food can be predicted on the basis of DEHA migration.

The predicted R346 migration into a variety of foods is compared to the measured migration in table 23. There is good agreement between the predicted and measured levels of R346 migration for the high fat content foods, that is cheese, egg mayonnaise sandwich and peanut biscuits, but poor agreement for low fat foods, that is chicken breast and swiss roll. The under estimation of the level of R346 migration into the chicken breast and swiss roll may be due to the polar nature of the surface of the food. Ruuska *et al.* found that the global migration from PVC milk tubing into water was considerably greater when the tubing was plasticised with a polymeric plasticiser ($\bar{x}$ = 6·2 $mgdm^{-2}$) than with DEHP ($\bar{x}$ = 0·5 $mgdm^{-2}$) (146). The greater global migration was attributed to the migration of small polar oligomers of the polymeric plasticiser. Thus, it would appear that for polar foods or food surfaces the oligomers in peak four migrate into the food to a greater extent than DEHA. Therefore the prediction of R346 migration on the basis of DEHA migration and the relative migration of R346 oligomers into olive oil can only be made for fatty foods.

Table 23. Comparison of Measured and Predicted Levels of R346 Migration From PVC Cling Film into a Variety of Food.

Food Type	% Lipid	Measured Total R346 Migration	Predicted Total R346 Migration	Percentage †
Cheese Slice	33.5	0.41 mg	0.31 mg	78
Egg Mayonnaise Sandwich		0.36 mg	0.20 mg	61
Chicken Breast		2.0 mg	0.37 mg	18
Peanut Biscuit	25	4.17 mg	1.71 mg	41
Swiss Roll Whole	15	2.08 mg	0.24 mg	11
Swiss Roll Slice	15	1.33 mg	0.21 mg	20

† The predicted total R346 migration expressed as a percentage of the total R346 migration found.
The percentage lipid was determined as the mass of lipid solvent extracted from a known mass of food.
Levels of R346 migration were determined in chapter six.

CHAPTER 8

CONCLUSIONS AND FURTHER WORK

8.1 PLASTICISER CONTAMINATION OF FOOD RESULTING FROM THE DOMESTIC AND RETAIL USE OF FLEXIBLE FILMS

8.1.1 CONCLUSIONS

DEHA plasticised PVC cling film was the only plasticised film available for domestic use. The survey of retail packaging material (chapter 3) revealed that three types of flexible films were in retail use, DEHA plasticised PVC cling film, PVDC plasticised with DBS or ATBC and RCF plasticised with two or more phthalate ester plasticisers.

The level of plasticiser contamination varied greatly with plasticiser and food type. The following levels were measured; DEHA 0·3-207 $mgkg^{-1}$ (domestic use), 0·04-72·8 $mgkg^{-1}$ (retail use), ATBC 1·3-7·7 $mgkg^{-1}$, DBS 2·3-137 $mgkg^{-1}$, DBP 0·5-30·8 $mgkg^{-1}$, DCHP 0·2-19·8 $mgkg^{-1}$, BBP 1·0-15·0 $mgkg^{-1}$ and DPOP 0·2-9·4 $mgkg^{-1}$.

The level of plasticiser contamination was dependant on a number of factors. These included: the fat form, whether the fat is concentrated at the surface or dispersed throughout the food, and content of the food; the length and temperature of exposure; and the manner in which the food was wrapped, that is the extent the film is stretched when it is applied, whether the food is completely over-wrapped in film, over-wrapped on a polystyrene tray or a deep sided tray, where the film is not intended to come into direct contact with the food, and whether there is over-wrapping of the film.

8.1.2 FURTHER WORK

The dietary intake level of DEHA calculated from the above and other work (106,107) was considered to be unacceptably high (117). As a result film manufacturers produced cling films with lower plasticiser migration into food and some retailers changed to non-plasticised plastic packaging material. The effect of these changes on the dietary intake of plasticisers is currently being assessed by MAFF. Migration experiments with low migration cling film and the estimation of plasticiser contamination resulting from the retail use of plasticised packaging material are in progress. The results of this work are to be published shortly (147).

8.2 MIGRATION CHARACTERISTICS OF POLYMERIC PLASTICISERS

8.2.1 CONCLUSIONS

The method of analysis for polyester plasticisers developed is applicable to a wide range of food types and PVC films plasticised with either polyester plasticisers or polyester and monomeric plasticisers.

The migration of the polyester plasticiser Reoplex 346 (R346), which is a copolymer of 1,3-butandiol and adipic acid, was between three and twenty times lower than that of DEHA, under identical exposure conditions. This reduction in migration may be attributed to the greater molecular weight of R346 compared to DEHA. R346 migration into fatty foods is inversely proportional to the square of molecular weight of the individual oligomer.

The level of R346 migration into fatty food can be accurately predicted from the molecular size distribution of R346 oligomers migrating into olive oil and the migration of DEHA into the food (under identical exposure conditions) under consideration. The predicted migration was within a factor of 2·5 of the measured migration for cheese, egg mayonnaise sandwich and peanut biscuits.

R346 migration into polar food predicted on the same basis was typically a factor of 7 smaller than the measured migration. The under-estimation of R346 migration is probably due to the small oligomers migrating to a greater extent into polar food than fatty food. Thus, the molecular weight distribution of R346 migrating into olive oil is not representative of the molecular weight distribution of R346 that migrates into polar foods.

8.2.2 FURTHER WORK

Determination of the molecular size distribution of R346 migrating into a polar food, such as milk or cream, may enable the level of R346 migration into polar foods to be predicted.

Structural elucidation of the oligomers that migrate into food would provide additional information for the prediction of polyester plasticiser migration into food. This information would also assist toxicologists assess the health risk that migration of R346 and adipate based polymeric plasticisers in general represents.

8.3 MATHEMATICAL MODELLING OF THE MIGRATION OF DEHA FROM PVC CLING FILM INTO FOOD

8.3.1 CONCLUSIONS

The mathematical model of additive migration from polymers into food proposed by Till *et al* (124) accurately predicates the migration of DEHA from PVC cling film into food. The predicted level of DEHA migration into cheddar cheese exposed for 1, 5 or 7 days at 5°C was within a factor of 1·5 of the measured migration.

The diffusion coefficient of DEHA in cheddar cheese is 3×10^{-8} cm^2s^{-1} at 25°C and $1{\cdot}5 \times 10^{-9}$ cm^2s^{-1} at 5°C. The partition coefficient of DEHA between cheddar cheese and PVC cling film is 0·58 at 25°C and 0·7 at 5°C. The value of both the diffusion coefficient of DEHA in food (D_f) and the partition coefficient of DEHA between food and PVC cling film are dependant on food type. The diffusion coefficient is particularly sensitive to variations in the composition and physical nature of the food. This has important implications for the prediction of DEHA migration as Till's model is sensitive to changes in the value of D_f. The dependence of the partition coefficient on the food type is of less importance as the model is relatively insensitive to changes in the value of the partition coefficient.

The diffusion coefficient of DEHA in PVC is independent of the food in contact with the film. Therefore the migration of DEHA from PVC cling film into food is Class II in nature.

8.3.2 FURTHER WORK

Determination of the diffusion coefficient of DEHA in food with different fat content, such as butter and yoghurt, would provide information on the relationship between fat content and the value of the diffusion coefficient. This information could then be used to estimate the diffusion coefficient of DEHA in any food. The migration of DEHA into any food could then be predicted using Till's model.

Information on the relationship between fat content and diffusion coefficient might permit the diffusion coefficient of other plasticisers in food to be estimated. These values could then be used in conjunction with Till's model to predict plasticiser migration into food. This would reduce the number of migration experiments required for dietary intake calculations and assist in identifying plasticisers most likely to migrate into food.

APPENDIX

The listing of the Mallard-80 BASIC computer program used to calculate DEHA concentration profiles is given below.

```
10 REM File Name : DEHA
20 REM This programe calculates the concentration profile of
 any diffusant from an extended source of limted extent.
30 REM The equation used is C = Co/2 ((erf(h-x)/2*sqr(Dt)) +
 ((erf(h+x)/2*sqr(Dt))
40 INPUT "Enter the initial concentration Co in g/cm3 ";Co
50 INPUT "Enter the half width of the concentration step (h)
 in centimetres ";h
60 INPUT "Enter the diffusion cofficient (D) in cm2/s ";D
70 INPUT "Enter the time of exposure (t) in seconds ";t
80 INPUT "Enter the starting value for x in centimetres (x i
s the distance from the orgin)";xm
90 INPUT "Enter the outer limit of x you require in centimet
res ";xn
100 INPUT "Enter the size of step you wish to consider in ce
ntimetres";p
110 LPRINT "The concentration of a diffusant from a slice of
 cheese with a half-width of ";h" cm with a diffusion coeffi
cient of ";D
"cm2s-1 and an initial concentration ";Co" gcm-3 left for ";
t" seconds is :-"
120 PRINT
130 LPRINT
140 LET nd=1+(xn-xm)/p
150 DIM v(nd)
160 a = 2*(SQR(D*t))
170 g = 0
180 FOR x = xm TO xn STEP p
190 z = (h-x)/a
200 GOSUB 350
210 v(g)=i
220 z = (h+x)/a
230 GOSUB 350
240 LET v(g)=Co/2*(v(g)+i)
250 PRINT ROUND(x,3);TAB(9);"C=";v(g);"g/cm3"
260 LPRINT "x =";ROUND(x,3);TAB(15);"C =";v(g);"g/cm3"
270 IF g=0 THEN q=v(g):y=v(g):GOTO 300
280 IF q<v(g) THEN q=v(g)
290 IF y>v(g) THEN y=v(g)
300 LET g=g+1
310 NEXT x
320 LPRINT
330 PRINT
340 END
350 REM Simpson's rule estimate of erf(z)
360 LET n=2
370 LET b=z/2
380 LET i=1
390 FOR j=1 TO n-1
400 LET i=i+2*(1+(j AND 1))*EXP(-j*j*b*b)
410 NEXT j
420 LET i=2*b/3/SQR(3.1415926£)*(i+EXP(-z*z))
430 IF ABS(i-k)>0.000001 THEN b=b/2:n=2*n:k=i:GOTO 380
440 RETURN
460 LPRINT CHR$(27);CHR$(108);CHR$(5)
470 LPRINT CHR$(27);CHR$(81);CHR$(65)
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